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Topical Antimicrobials for Burn Infections – An Update

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Abstract

The relentless rise in antibiotic resistance among pathogenic bacteria and fungi, coupled with the high susceptibility of burn wounds to infection, and the difficulty of systemically administered antibiotics to reach damaged tissue, taken together have made the development of novel topical antimicrobials for burn infections a fertile area of innovation for researchers and companies. We previously covered the existing patent literature in this area in 2010, but the notable progress made since then, has highlighted the need for an update to bring the reader up to date on recent developments. New patents in the areas of topically applied antibiotics and agents that can potentiate the action of existing antibiotics may extend their useful lifetime. Developments have also been made in biofilm-disrupting agents. Antimicrobial peptides are nature's way for many life forms to defend themselves against attack by pathogens. Silver has long been known to be a highly active antimicrobial but new inorganic metal derivatives based on bismuth, copper and gallium have emerged. Halogens such as chlorine and iodine can be delivered by novel technologies. A variety of topically applied antimicrobials include chitosan preparations, usnic acid, ceragenins and XF porphyrins. Natural product derived antimicrobials such as tannins and essential oils have also been studied. Novel techniques to deliver reactive oxygen species and nitric oxide in situ have been developed. Light-mediated techniques include photodynamic therapy, ultraviolet irradiation, blue light, low-level laser therapy and titania photocatalysis. Passive immunotherapy employs antibodies against pathogens and their virulence factors. Finally an interesting new area uses therapeutic microorganisms such as phages, probiotic bacteria and protozoa to combat infections.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

1. INTRODUCTION

The relentless rise in antibiotic resistance is widely forecast to become one of the major medical problems in the 21st century [1]. Predictions of the imminent arrival of the end of the antibiotic era are regularly made [2, 3], and fears have been expressed that serious injuries or large scale burns could become the portal of entry for life threatening infections, as was the usual case before antibiotics were discovered. Widespread overuse and abuse of antibiotics [4], together with inappropriate use of antibiotics in livestock feedstuffs [5], are partially blamed for this worrying development, but in reality the rapid evolution of microorganisms together with the phenomenon of lateral gene transfer between species may have made the development of resistance inevitable [6]. There is also concern about the rapid global spread of resistant strains as exemplified by New Delhi metallo-β-lactamase - mediated carbapenem resistance [7]. Despite major efforts to discover new antibiotics remarkably few truly novel compounds have been discovered in recent times [8].

Burns are particularly susceptible to infection for several reasons. The disruption of the epidermal barrier combined with the denaturation of proteins and lipids provides a fertile environment for microbial growth [9]. Furthermore, a complex cascade of biochemical events leads to a “systemic apoptotic response” and thence to immunosuppression that abrogates the normal self-defense mechanisms that would fight infection [10]. The difficulties faced by systemically administered antibiotics in reaching the damaged tissue with its compromised blood circulation, have encouraged the use of topically applied antimicrobial products [11, 12].

In a previous review in this Journal [13] published in 2010 we covered issued patents and patent applications on topical antimicrobial agents that had been proposed to be used to prevent and treat burn infections. However this field has continued to be a remarkable source of invention and innovation in the antimicrobial arena, and we feel an update is now required to bring the reader up to date. The previously covered agents are summarized in tables in the present review, allowing the newly issued patents and publications to be covered in some detail while allowing easy comparison with already known agents and approaches.

2. MICROBIOLOGY OF BURN WOUND INFECTIONS

As we stated previously, burn wounds are susceptible to infection due to various reasons. Thus, a long list of microorganisms (including the species present in our normal skin microflora) has been observed to colonize burn wounds, and in some cases to cause serious infections. A list of burn wound pathogens with their relative abundances is given in Table 1.

3. BURNS AND ANIMAL MODELS OF BURN INFECTION

Skin is the first line of defense as it acts as a physical barrier against microbial invasion. Impairment of this important defensive structure renders the body particularly susceptible to infections.

Burn injury can cause substantial damage to the skin and compromises its defensive role. Burns destroy the cutaneous barrier, including the vascular supply, and this damage can lead to an impairment of the immune system. Moreover, the burned area is rich in bacterial nutrients and is thus significantly prone to infection, which remains as one of the major causes of morbidity and mortality in burn patients. The area of the body that is burned is the most important factor in deciding outcomes in patients, with burns over 50% of the body often proving fatal (see Fig. (1).

The motivation to develop animal burn wound models is to study the course and treatment of various infections, and to study the healing processes of established wounds. In recent decades several animal burn wound models have been developed. The animals used in these models include several different rodents, as well as rabbits and pigs.

The different classes of severity of burns are commonly classified according to the depth of the injury, which in turn depends on the length of time the skin is exposed to heat [14]. The size of the wound is also an important factor for classifying burns and burn models. Additionally, if an infection is established in the wound, the number of inoculated bacteria, the method of inoculation and the virulence of the strains also affect the severity of the infection. In the models covered in this review, the size of the burn wounds ranges from 5% to 50% of total body surface area (TBSA) and the wounds have different depths. Fig. (2) shows a few of the animal models of burns used to study infections.

In 1968, Mason and Walker developed the first burn model in rats that used boiling water to inflict a scald injury [15]. The complete procedure used in this model has been described in the previous review [13]. Burns were inflicted on the shaved dorsum of anesthetized animals. Each animal was placed in a fixed-area shield and the dorsum was immersed in boiling water. This procedure produces a uniform partial-thickness burn (three seconds of exposure) or a full-thickness burn (ten seconds of exposure), covering approximately 30% TBSA. This model has been widely used for studying burn infections with pathogens such as *Pseudomonas aeruginosa* and potential treatments [16], bacterial translocation and intestinal atrophy after thermal injury [17], burn sepsis [18, 19], candidiasis after thermal injury [20], and gene therapy [21, 22].

Bjornson *et al*. [23] developed a similar model using guinea pigs to study burn wounds infected with *Staphylococcus aureus*, *P. aeruginosa* and *Candida albicans*. The burn injury covered a 60 cm^2 area on the dorsum and was produced by immersing the area in boiling water for thirteen seconds. After one hour the animal received a second burn injury. After the wounds were established, they were infected with the pathogens. It has been reported that the skin of guinea pigs reacts in a similar manner to human skin when exposed to burn injury, thus this model may be used to mimic clinical burn injuries. The model of Orenstein *et al*. [24] also used a guinea pig where the animal was subjected to a fifteen second metal plate application, which was preheated to 150°C. Fifteen minutes after the formation of a third-degree burn, the wound was inoculated with *S. aureus*.

A thermal injury burn model using mice was established by Stieritz and Holder [25]. The mice in the model were shaved and ethanol was applied to the shaved dorsum. Following the

ethanol application, the substance was ignited and allowed to burn for ten seconds, which created a wound comprising about 30% of TBSA. The model has been used to study the pathogenesis of *P. aeruginosa* [25, 26] and *Klesbiella pneumonia* [27] in murine burn wounds where the bacteria are inoculated subcutaneously.

Katakura *et al*. [28] created a novel burn wound model that induced thermal injury by exposing the shaved back of the mouse to a gas flame for nine seconds. The flame was focused on a specific area of the dorsal skin by putting an insulated mold around the flame which formed a third degree burn covering 15% of TBSA. Katakura *et al.* used the model for studying the pathogenesis of MRSA but the model has been used in other studies as well [29–31].

Several burn models have been established which create the burn by applying the heat directly to the skin, for instance by putting the skin in contact with pre-heated objects. The model by Stevens *et al*. used two brass blocks that were preheated to 92–95°C and then applied on either side of an elevated skin fold, subsequently causing a burn wound that was approximately 5% of TBSA. Afterwards, the wound was infected with an intradermal injection of *P. aeruginosa* [32]. This model has been used in testing the efficacy of photodynamic therapy PDT) against burn infections [33–36]. Manafi *et al*. [37] introduced a novel burn model, which used a heated metal block that was applied on the dorsal side of mice to produce burns that were 10% of TBSA and subcutaneously injected *P. aeruginosa*. Kumari [38] used a similar model but infected the wound by topically inoculating the bacteria.

Kaufman *et al*. (38) proposed a new burn model using guinea pigs that were subjected to a deep partial thickness burn by applying a pre-heated aluminum cylinder at 75°C to the back of the animal. In another model, Branski *et al*. (39) used an aluminum bar which was heated to 200°C and caused a full-thickness burn injury covering approximately 15% of the TBSA of the pig. Similarly to the previous model, a porcine burn model was described by Middelkoop *et al*. [39] that incorporated the use of brass blocks at 170°C to create a superficial contact burn when applied for 10 seconds, or deep partial thickness burn when the blocks were held on the skin of the animal for 20 seconds. Knabl *et al*. [40] used rabbits to study the effects of burn injuries by inflicting thermal damage with a soldering iron at 80°C for 14 seconds.

Miscellaneous methods of inflicting thermal injury have also been incorporated in several animal models using rats and pigs. Suzuki *et al*. [41] constructed a glass chamber through which water at a pre-determined temperature circulated. Rats could be pressed against the chamber and sustained the injury by absorbing heat through the glass. The model provided linearity regarding the severity of the injury vs the following variables: temperature of water, pressure applied on the animal when pressed against the chamber, and exposure time. Bahar *et al*. [42] immersed a lint cloth in boiling water then applied the cloth on the rat dorsum to create burn wounds which could be superficial or deep depending on the length of the procedure time. Gurfinkel *et al.* [43] used a radiant heater set at 400°C and exposed the shaved skin of pigs and rats to the heater for twenty seconds to establish wounds that comprised 30–50% of TBSA [44].

4. TOPICAL ANTIBIOTICS

Because the microcirculation in burned skin is more or less destroyed, orally or systemically (intravenously) administered antibiotics are relatively ineffective, as the compounds cannot reach the actual site of the infection in a sufficient concentration. For this reason various preparations and formulations of antibiotics for topical application have been developed and patented. It should be pointed out that it has been suggested that topical antibiotics may be more likely to lead to development of resistance than systemic antibiotics [45]. Table 2 lists the topical antibiotics covered in the original review.

4.1. Firmocidin

Firmocidin (Fig. 3A) is a novel antimicrobial molecule that has shown broad-spectrum activity against a number of Gram(+) and Gram(−) bacteria including MRSA, as well as fungi. Gallo *et al.* [46] patented this new antimicrobial agent and its method of preparation in 2012. Firmocidin is isolated from the culture supernatant of a clinical strain of *Staphylococcus epidermidis* (MO34). *S. epidermidis* is an important species in the normal skin microflora due to its production of antimicrobial peptides called phenol-soluble modulins (PSMs) which benefit the skin by preventing the colonization of pathogenic bacteria, and the presence of lipoteichoic acid which suppresses inflammation during wound healing. The normal skin microflora is therefore an important factor that helps prevent the colonization of pathogenic organisms on the skin surface [47].

Firmocidin may be useful due to its high antimicrobial effectiveness against most common burn wound pathogens such as MRSA, *S. aureus*, group A streptococcus (GAS) and group B streptococcus (GBS) and its low toxicity against normal skin microflora unlike most other antibiotics. Thus, the selective nature of firmocidin to kill pathogenic species while permitting survival of *S. epidermidis*, allows the latter species to produce antimicrobial peptides which have a role in the innate defense system of the skin. For atnibiotic treatment of wound infections, the route of administration is mainly topical but the formulation could be applicable for other routes of administration. In some embodiments, firmocidin is combined with additional therapeutic agents including antibiotics, steroids and antifungals, thus achieving a greater efficacy [46].

4.2. Vancomycin

Vancomycin is classified as a glycopeptide antibiotic which is produced by the bacterium, *Amycolatopsis orientalis* [48]. It inhibits the cell wall biosynthesis of resistant-type bacteria and/or Gram(+) bacteria including staphylococci, and most importantly, is considered as a first-line treatment for MRSA infections. It can be administered by intravenous, oral and topical routes [49]. In case of a localized burn wound infection, topical vancomycin should be preferred over systemic formulations because of the high hepatorenal toxicity of systemic vancomycin and, as Vingsbo *et al.* [50] have shown, the comparative ineffectiveness of systemic vancomycin in the reduction of bacterial load in the wound. Berman [51] invented an anti-MRSA bactericidal topical gel containing vancomycin that is specially suitable for the promotion of wound healing by supplying a moist environment at the wound site. The invention is a topical form of vancomycin that has sustained potency and stability. A prior

relevant study described a dressing containing vancomycin with a stability of less than 30 days [52] while vancomycin in Berman's topical gel sustained its potency and stability for more than 2 years. Other than the main antimicrobial agent that is selected to be a therapeatucilly effective amount of vancomycin defined by the amount that can prevent microbial growth of MRSA on the wound site, the composition may contain additional antibacterial agents, gel forming agents and preserving agents.

4.3. Fusidic Acid

Fusidic acid (FA, Fig. 3C) is a bacteriostatic antibiotic that inhibits protein synthesis, primarily in Gram(+) bacteria. It was isolated from a fungus, *Fusidium coccineum* sp. in the 1960s [53]. Despite being effective against many pathogens such as *Staphylococcus* spp., *Streptococcus* spp. and *Corynebacterium* spp., and being approved in Europe and Asia, the drug has never been approved by the US Food and Drug Administration [54]. Moreover, interest in the drug declined with the development of other, more potent antibiotics. However, interest in FA rose again as strains of bacteria resistant to these drugs, such as MRSA, emerged [54].

In various studies FA exhibited desirable therapeutic effects against burn wound pathogens *in vivo* and *in vitro* [55]. In a study done by Ulkur *et al.* [56] on MRSA contaminated full-thickness rat burn wounds, MRSA was eradicated in burn eschars that were treated topically by 2% fusidic acid composition, while silver impregnated wound dressing and chlorhexidine acetate treatments achieved significantly lower decreases in the bacterial load. In another study Vingsbo *et al*. [50] observed significant log reductions of MRSA, 2.9 log after 3 days, 4.2 log after 6 days, in murine skin wounds with topical FA treatment. Vanangamudi *et al.* [57–61] patented numerous creams containing FA that can be applied topically to bacterial skin infections, including burn wound infections. Moreover the patented creams may further contain a steroidal agent for anti-inflammatory purposes, chitosan as a biopolymer agent for wound healing purposes and an antifungal agent for increased broad-spectrum antimicrobial effect.

4.4. Usnic Acid

Usnic acid (UA, Fig. 3D)) is a dibenzofuran derivative antibiotic that is found in nature as a metabolite of lichen. It was observed that the compound possesses intrinsic antimicrobial activities which are particularly effective against Gram(+) planktonic and biofilm-forming bacteria [62]. Furthermore, in a study conducted by Lauterwein *et al.* [63] methicillin and/or mupirocin resistant strains of *S. aureus* were found to be susceptible to the compound suggesting UA could be an alternative to several commonly used antibiotics. In another study, UA was able to inhibit the growth of various *S. aureus* strains at lower minimum inhibitory concentration (MIC) values than antibiotics such as oxacillin, clindamycin and gentamicin, while also showing synergism when used in combination with these antibiotics [64]. UA is believed to exert its antimicrobial potency by sensitizing bacteria to salts in the surrounding medium thus causing membrane damage to susceptible bacteria [65]. Also, UA possesses some wound healing and anti-inflammatory properties, further suggesting its possible use in burn wound treatment. A study by Nunes *et al.* [66] showed that collagen-

based films which contained liposomal UA performed better than non-UA collagen-based films as a dressing in dermal burns.

Eady *et al*. [67] acquired a patent for topical formulations containing UA or usnate with a metal salt that can be used for treating infected burn wounds. The metal salt is either a copper or a bismuth salt; both of which have been previously described as antimicrobials. Thus the constituents of the patented formulation display synergism in reducing bacterial loads. However, UA is poorly water soluble which may pose some problems in burn care [68]. Nevertheless, this drawback of the compound may be overcome, as in a study by Francolini *et al.* [69] that showed that an UA-polyacrylamide complex performed better as an antibacterial agent against *S. epidermidis* than did free drug UA and the complex was highly soluble in water.

4.5. Tetracyclines

Tetracyline antibiotics having a broad-spectrum of bacteriostatic activity were first isolated in 1948 [70]. Their mechanism of action involves ribosomal binding leading to inhibition of protein synthesis. Many tetracycline compounds are affected by crucial problems of resistance emerging among many clinically important bacteria, and also tend to have highly unstable structures [71, 72]. The susceptibility of these compounds to oxidation and their tendency to degrade in general, result in chemically unstable topical formulations. Thus, a lot of studies have been done in order to formulate chemically stable complexes and chemically modified forms of the tetracycline molecule to overcome the aforementioned problems. By definition, “chemically stable” is showing substantially none or minimal breakdown from oxidation in 3 days after mixing with the agent or the carrier while kept at 25°C minimum. According to recent patents and studies, foamable compositions have exhibited usefulness in this regard [73, 74]. Various recent patents can be found in Table 4 and structures in (Figs. 3F and 3G).

4.6. Aminoglycosides

Aminoglycosides are effective against aerobic Gram(+) and Gram(−) bacteria. Prior [75] invented a method for treating infected wounds, mainly diabetic ulcers but the treatment is also mentioned to be applicable for burn wounds. The method of treatment involves a combination of the topical administration of one or more aminoglycoside antibiotics at the infection site and the preferably systematic administration of one or more antibacterial agents. In a particular embodiment the aminoglycoside antibiotic is selected to be gentamycin sulfate, a broad spectrum antibiotic that is useful for the treatment of infected burn wounds and effective against a number of marked burn wound pathogens [76]. The agent is uniformly dispersed in a type-I collagen matrix. The method of treatment has been observed to be bactericidal against most of aerobic Gram(−) and Gram(+) bacteria and facultative anaerobic Gram(−) bacteria like MRSA.

In another invention, Coates *et al.* [77] combined an aminoglycoside antibiotic with a pyrroloquinoline compound. The synergism between pyrroloquinoline compounds and various aminoglycoside antibiotics such as kanamycin, gentamycin and tobramycin has been seen *in vitro* tests. For example, a clinical isolate of *P. aeruginosa* was exposed to specific

amounts of tobramycin and a pyrroloquinoline compound separately after being cultured. While the compounds alone showed no or slight activity against *P. aeruginosa*, in combination they resulted in a rapid elimination of the bacteria. The composition is proposed as a treatment of burn wound infections caused by a list of Gram(+) and Gram(−) bacteria and fungi of importance. Additionally, in another patented invention by Hu *et.al*, a pyrroloquinoline is used in combination with a beta-lactam antibiotic, mupirocin or clorhexidine. Synergism was observed in the same manner.

4.7. Nubiotics

Developed by Roderic M.K. Dale [78], nubiotics are a new class of drugs that are nucleotide derivative compounds which show antimicrobial activity. They have been developed as a possible new approach to combating MDR bacteria. The antimicrobial mechanism has not been completely elucidated but preliminary studies indicated that nubiotics may be effective against burn wound infections. Nubiotics can be administered in a variety of routes including topical and in different forms such as liposomal or in hydroxypropyl methylcellulose (HPMC) coated vehicles. HPMC is a polymer that enables the sustained release of the drug. Although several nubiotics have been developed, Nu-2 proved to be the most efficacious treatment for fatal *P. aeruginosa* burn infections in mice. The coating has been reported to increase the survival rate of mice when HPMC coated Nu-2 is compared to the non-coated versions [79].

5. ANTIMICROBIAL RESISTANCE INHIBITORS AND DRUG POTENTIATORS

Several different biological mechanisms that confer antimicrobial resistance have evolved in microorganisms [80]. These mechanisms can either: chemically modify the antimicrobial agent; render it inactive through physical removal from the cell; or modify the target site so that it is not recognized by the antimicrobial as shown in (Fig. (4). Resistance may be an inherent trait of the organism (e.g. a particular type of cell wall structure) that renders it naturally resistant, or it may be acquired by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source.

5.1. Alginate

Alginic acid (alginate, Fig. 3B) is a widely used pharmaceutical excipient, which is isolated from the cell wall of marine brown algae and some bacteria such as *Azotobacter vinelandii* and *P. aeruginosa* [81, 82]. When isolated naturally, alginate polymers (copolymers of homopolymeric blocks of 1–4 linked β-D-mannuronic acid and C-5 epimer-α-L-guluronic acid residues) have high molecular weights which are lowered by chemical or enzymatic breakdown while being processed for pharmaceutical usage. Alginate had been previously used as a binder and disintegrating agent in tablets and capsules [83], a stabilizing agent, antacid, human appetite suppressor [84] and as a wound dressing component particularly for burn wounds [82, 85].

Dessen *et al.* [81] invented a method for treatment of *Acinetobacter* infections which integrated the use of alginate oligomers (AO) in combination or conjunction with antibiotics, particularly macrolides such as azithromycin. AO have been found to enhance the efficacy

of antibiotics against the genus *Acinetobacter*. According to the data obtained from an *in vitro* assay conducted by the inventors, *A. baumannii* growth could be completely inhibited by a combination of 4μg/ml azithromycin and 2% or more AO whereas neither 4μg/ml nor 8 μg/ml of azithromycin by itself was sufficient enough to inhibit the growth. Another experiment was done using azithromycin combined with one other antibiotic and AO on an MDR strain of *P. aeruginosa* and AB. The addition of AO was able to decrease MIC values of the compositions against both bacteria while achieving greater MIC reduction against AB. The patent holds great significance for infected burn wounds where *Acinetobacter* infections can be prevented or treated more efficiently by the topical administration of the said combination that might be incorporated into creams, gels, ointments, transdermal patches, wound dressings and the like.

Another invention by Dessen *et al.* [86] involves reversing resistance of an MDR bacterium to at least one antibiotic by treating the bacterium with the combination of AO and the antibiotic. The invention is applicable to burn wound infections because the composition is mainly administered topically, and can target a number of clinically important MDR wound pathogens such as but not limited to *Pseudomonas*, *Acinetobacter* and MRSA. The combination of AO and the antibiotic can be incorporated into the same topical forms mentioned regarding Dessen's other patent. An assay comprising PA, AB and KP that were treated with various antibiotics from different groups administered in combination with AO resulted in decreased MIC values in proportion to the increasing concentrations of AO. More specifically, the potentiation was observed in every combination of bacteria and macrolides. Additionally, AO given with specific antibiotics have been found to inhibit biofilm formation of such MDR bacteria, thus the tolerance of burn wound biofilms to antibiotic agents is reduced [87].

Topical alginate shows potentiation not only for antibiotics but also for antifungals. Enhancing the efficacy of anti-fungals is essential because of two reasons: (1) acquired resistance of fungi which is observed less rapidly compared to bacteria [88]; (2) high toxicity of antifungals to human cells which necessitates using lower amounts of antifungals in treatments [89].

Onsoyen *et al.* [90] patented a method of treating a fungal infection by using AO with one or more antifungal agents, preferably selected from the allylamine, azole, echinocandin or polyene groups. In an assay testing the effect of alginate on the antifungal efficacy against *Aspergillus* species, a reduction is observed in MIC values of the antifungal agents. It is believed that AO might be a way of reducing the tolerance or increasing the susceptibility of fungi to antifungals.

5.2. Polyamines

As mentioned earlier in this article, microorganisms might increase the number and/or the activity of their efflux pumps as an antimicrobial resistance mechanism. Inhibiting these efflux pumps, which are transport proteins located in the plasma membranes of microorganisms and pump out the toxic materials including antibiotics, is a way of overcoming antimicrobial resistance [91].

Nelson *et al.* [92] developed a method of treating infections caused by bacteria including multidrug resistant burn wound pathogens by using polyamine compounds that inhibit efflux pumps of the pathogenic microorganisms. Polyaza-alkanes, polyaminoalkanes, or mixed poly(aza/− amino)alkanes are examples of the polyamine compounds. In a study done by Dela Vega *et al.* [93], different types of polyamines were tested on *E. coli* exposed to beta-lactam antibiotics and exhibited the inhibition of efflux pumps. In another study, Kwon *et al.* [94] reported similar data that addition of spermine, a polyamine, to the antibiotic treatment reduced the growth rate of *E. coli*, and gave total inhibition when added in higher concentrations. The experiment was repeated for *S. aureus* strains and the MIC values of beta-lactams were found to be decreased dramatically in the presence of a polyamine, while spermine also increased SA susceptibility to cloramphenicol, polymyxin B and tetracyclines. David and Dutta filed a patent [95] on the use of polyamines with varying chain-lengths to bind to and permeabilize intact Gram negative bacterial membranes. The compounds were found to possess significant antimicrobial activity mediated via permeabilization of bacterial membranes. Homologated spermine, bis-acylated with C8 or C9 chains was found to profoundly sensitize *E. coli* to hydrophobic antibiotics such as rifampicin.

5.3.Methylsulfonylmethane (MSM)

MSM (Fig. 3H) is an organosulfur compound which is a derivative of dimethyl sulfoxide (DMSO) and is marketed as a dietary supplement mainly for the treatment of osteoarthritis [96]. However, Benjamin *et al.* [97] unexpectedly discovered that MSM may have an important role in treating both drug-sensitive and drug-resistant microorganisms. MSM was found to sensitize MRSA to drugs it was resistant to. Contact of the burn wound pathogen with MSM and the drug can be implemented by topical administration. MSM may be incorporated into the compositions between the range of 0.01% and 20% by weight.

6. BIOFILM DISRUPTING AGENTS

Biofilms describe the life-style of groups of microorganisms that attach to surfaces of many kinds (including human tissues) and proliferate. A biofilm might be monolayer or multilayer depending on its phase. A multilayer biofilm involves cell-to-cell attachment besides surface attachment and contains an extracellular matrix consisting of protein, exopolysaccharide (EPS) and sometimes DNA which are all produced by the microorganisms embedded in the biofilm [98–100]. The stages of biofilm formation and subsequent dissolution is shown in (Fig. (5). Essentially, most pathogens are capable of forming biofilms.

Biofilm formation is thought to be the preferred lifestyle of pathogens, particularly bacteria. CDC and NIH have stated that biofilms account for 65% of nosocomial infections and 80% of all known infections [101]. Biofilm formation provides a number advantages to microorganisms such as protection from a harsh environment, increased survival caused by gene up- or down-regulation, reduced susceptibility to antimicrobials [102, 103], neutralization of some antibiotics by EPS [104] and a barrier against the host immune system [9, 105].

It is well known that biofilm-forming pathogens are important in burn wound infections [9]. *In vivo* experiments on various animal burn models demonstrated that formation of a mature

biofilm takes 41.5 to 72 hours [9, 106] while in *in vitro* experiments it required 10 hours [107]. For the evaluation of biofilms in burn wounds, various burn-biofilm models have been established such as Zurich burn-biofilm model [106]. Biofilm disrupting and inhibiting agents have emerged as a relatively new method of prevention and treatment of burn wound infections.

6.1. 2-aminobenzimidazole

Compounds containing the 2-aminobenzimidazole (2-ABI) structure were discovered as natural products in certain marine sponges, that were responsible for the resistance of these sessile organisms to the growth of marine biofilms [108]. 2-ABI is a heterocyclic aromatic compound with an amino group attached to the 2nd carbon of the five-membered ring as found in the sponge metabolite bromoageliferin [109]. Before Blackwell *et al.* [110] described and patented 2-ABI compounds that can inhibit or disperse biofilms of Gram(−) bacteria particularly *P. aeruginosa*, 2-ABI was known to be active against the biofilms of mainly Gram(+) bacteria. This statement was supported by a study of Rogers *et al.* [111] on the spectrum and mechanism of activity of 2-ABI. According to the conclusion drawn from their findings, which has partially been refuted by the experiments of Blackwell *et al.*, the biofilm formation of *P. aeruginosa* and multidrug resistant *A. baumannii* could not be inhibited by 2-ABI whereas an antibiofilm activity was observed just against Gram(+) bacteria tested. In fact, a therapeutically effective amount of 2-ABI exhibited an inhibiting activity on Gram(−) bacteria as well.

The assays of the compositions patented by Blackwell *et al*. [110] showed that 2-ABI treatment reduced the activity two types of quorum sensing receptors belonging to *P. aeruginosa*. Thus, quorum sensing inhibition might be the mechanism of inhibition of the biofilms of Gram(−) bacteria. The mechanism of inhibition observed in the experiments of Rogers *et al.* was totally different and indicated a Zn(II)-binding mechanism.

6.2. Polyanionic Compounds

For the topical treatment of wound infections and microbial biofilms, Hamerslag *et al.* [112] invented a combination of a polyanionic compound such as a polyphosphate and an antimicrobial agent. Said polyanionic compounds have the ability to chelate cations such as calcium, magnesium, manganese and iron [113], thus leading to the breakdown of the biofilm in an environment lacking these essential elements. The resulting situation leaves microorganisms “unprotected”, more susceptible to antimicrobials which are also included in the patented composition.

6.3. “Biofilm Destructor”

Gerard *et al.* [114] developed a “biofilm destructor” that receives a gas mixture containing ozone and oxygen via an entry port and delivers it through small needles that differ in length so that both the wound and interior parts of the biofilm are well exposed to the mixture. Ozone is considered an active antimicrobial against planktonic microbes whereas some studies such as Bialozewski *et al.* [115] demonstrated that oxygenozone mixture has relatively low antimicrobial activity against biofilms. The biofilm destructor comprises an ozone generator that generates ozone and oxygen at a predetermined ratio and a self-

adhesive treatment chamber, which should be in contact with the infection site. There is also a control unit disclosed in order to control the temperature, pH and ozone concentrations via a sensor. The invention presents a very effective way of administering the ozone/oxygen mixture to wounds in order to combat biofilms and we can consider it as a promising invention that can also be used for the delivery of various other antimicrobials to a wound site.

6.4. D-amino Acids

D-amino acids exhibit effectiveness in dispersing biofilms [116]. Studies have shown that the presence of D-amino acids prevents some clinically important pathogens such as *S. aureus* and *P. aeruginosa* from forming biofilms. In addition, D-amino acids are produced by bacteria themselves and induce biofilm disassembly [116, 117]. Losick *et al.* [118] acquired the patent for methods of treating biofilms using D-amino acids. The patented compositions are claimed to be active against the biofilms of a list of Gram(+) and Gram(−) bacteria. The compositions can be incorporated into wound dressings, gels, creams etc. The inventors tested the compositions on some common burn wound pathogens including *S. aureus* and *P. aeruginosa* and obtained positive results.

In another invention, Bottcher *et al.* [99] described compositions of polyamines combined with D-amino acids in order to treat biofilms. Polyamines are similar to D-amino acids with respect to their activity against biofilms. They also trigger disassembling of the biofilm, and norspermidine, a polyamine homologue of spermidine is produced by some microbes for the same purpose [119]. The invention was tested on *E. coli* as a typical burn wound pathogen.

6.5. DispersinB® Based Formulations

DispersinB® is an antibiofilm enzyme which is essentially soluble beta-N-acetylglucosaminidase similar to the product of the dspB gene [120]. It catalyzes the hydrolysis of the poly-N-acetylglucosamine (PNAG), one of the exopolysaccharides of the biofilm produced by prevalent burn wound pathogens such as *S. aureus* and *E. coli* [121, 122]. It is marketed by Kane Biotech Inc. as an antimicrobial for wound infections. Madhyastha *et al.* [120] invented compositions based on DispersinB® for the inhibition of growth and proliferation of biofilm-embedded microorganisms. The embodiments may contain a compound comprising DispersinB® as the biofilm dispersing agent, and an antimicrobial agent. The embodiments of the invention are applicable to both partial and full thickness burn wounds in the form of gels, wound dressings and alike. The spectrum of activity of DispersinB® includes many pathogens including but not limited to VRE, *S. epidermidis, S. aureus, K. pneumonia*, *Bacteriodes* and *Candida* species.

7. ANTIMICROBIAL PEPTIDES

Antimicrobial peptides (AMPs) are a group of naturally produced cationic molecules that comprise varying number of amino acid residues in an amphipathic formation. These molecules have been isolated from a diverse group of organisms which includes bacteria, amphibians, insects and mammals [123]. Mammalian AMPs are referred to as host defense peptides (HDPs); they are mainly found in skin, mucosal and immune cells and have

intrinsically biocidal effects. These endogenous molecules are capable of binding to numerous pathogens by exploiting their common motifs and therefore have broad-spectrum antimicrobial activities. The most well described mechanism of action of AMPs is by damaging cellular membranes of microbial cells but other mechanisms have also been proposed [124]. Moreover mammalian AMPs may have some immunomodulating properties and can improve angiogenesis, tissue repair, inflammation and adaptive immunity [125]. The expression of HDPs in tissues may be constitutive or may change according to different stimuli [126].

The role of the cathelicidin and defensin families as cutaneous AMPs have been extensively researched but many other peptides such as lactoferrin, lysozyme and histones are also regarded as significant HDPs [127]. Many of these HDPs (and other AMPs that are not found in the human body such as cecropin and bacteriocins) are being developed and modified for possible pharmaceutical uses in combating MDR pathogens. AMPs that were covered in the previous review are listed in Table 5.

7.1. Cathelicidins

Cathelicidins were the first group of AMPs found in mammalian skin; the peptide can be isolated from many cells such as keratinocytes, fibroblasts and neutrophils. The cathelicidin antimicrobial peptide (CAMP) gene expresses an inactive precursor protein in pre-pro-peptide form called hCAP-18 which is then cleaved by serine proteases into 2 functional molecules; cathelin and LL-37, a peptide sequence starting with 2 leucine residues and comprising 37 amino acids [125] (shown in Fig. (6). Cathelin functions as an antimicrobial and as a protease inhibitor [128]. LL-37 is an effective bactericide, virucide [129] and fungicide [130]. Furthermore, LL-37 can modify host immune responses by interacting with cell surface receptors such as Toll-like receptors, a class of receptors usually expressed in human innate immune cells, and G-protein-coupled receptors. Moreover, LL-37 serves as a chemotactic agent for immune cells and may potentiate immune mediators and other AMPs such as defensins thus inducing cytokine synthesis and further chemotaxis. Collectively the immunomodulatory effects of LL-37 have been called its “alarmin” function [127]. LL-37 molecules can be cleaved further to form several different AMPs [131, 132].

Several patents have been issued for cathelicidins and their derivatives. Stahle-Backdahl *et al.* [133] patented LL-37 for healing wounds and included a lipid bilayer that is claimed to reduce the cytotoxic effects of the peptide. Gemba *et al*. produced novel peptides with similar effects to LL-37 [134, 135]. Krieger *et al.* [136] patented indolicidin, a cathelicidin derivative peptide, which can be applied topically to treat burn wound infections. Indolicidin has broad and potent antimicrobial effects. The peptide has DNA binding properties and can interfere with its structure. It can also serve as a resistance inhibitor for aminoglycosides [137]. Modified analogs of indolicidins displayed increased antimicrobial efficacy and reduced hemolysis against human erythrocytes [138]. Omiganan is an indolicidin derivative that is being researched as a novel class pharmaceutical against burn wound pathogens [139, 140].

A study conducted by Kaus *et al*. regarding the levels of AMPs in epithelial cells collected from healthy, unburned skin (in close proximity to the burned area) and burned areas of the

skin concluded that LL-37 maintained similar expression levels in burned tissue compared to healthy tissue, while there is a significant decrease of LL-37 expression in unburned epithelial cells [126]. The study suggested the decrease may play a role in the pathogenesis of wounds.

In a study by Thomas-Virnig *et al.* keratinocyte progenitor cells were non-virally transfected with hCAP-18 genes and were tested as a live-tissue wound dressings for murine scald burns [141]. These transfected cells produced ~140 times more hCAP-18 protein than the control cells. The engineered tissue achieved significant antimicrobial efficiency, nearly 3 log, against MDR *A. baumannii* strains inoculated onto the wound site after 3 days. Although no wound healing effects were observed over the course of the study, the research suggests that the LL-37 dressing would stimulate re-epithelization and help wound closure over time. Centanni *et al.* patented a “human skin equivalent” that incorporated the methods of the aforementioned study [142]. The skin equivalent consisted of Near-Diploid Immortal Keratinocytes (NIKS) [143] expressing exogenous AMPs upon genetic modification. The AMPs are selected from cathelicidin class peptides. The modified NIKS can be used for treating wounds against infections and promoting their closure.

A similar study by Jacobsen *et al.* [22] tested the effects of various delivery methods of hCAP-18 and its products on rat scald burns. Topical administration of synthetic LL-37 peptide and adenovirus vectors containing hCAP-18 gene were compared for their efficacy in reducing bacterial load in burn wounds. Transient adenovirus therapy in the study aimed to transfect cells such as fibroblasts and keratinocytes, which should result in the up-regulation of hCAP-18 gene expression. The study showed significant increases in the production of hCAP-18 peptide in both cells upon transfection by the vector. Furthermore, cutaneous adenoviral gene therapy was significantly better than synthetic LL-37 administration in inhibiting growth of inoculated *P. aeruginosa*. Proteoglycan encoding adenovirus vectors have previously been patented as novel wound healing agents for cutaneous injuries [144].

7.2. Defensins

Defensins are cysteine-rich AMPs that have disulfide bonds in various arrangements. The nomenclature is based upon an arrangement from which 4 different classes of defensins can be named, but only α and β-defensins are found in humans [125]. Both classes of defensins have been isolated from skin, mucosal and immune cells, which are constantly exposed to pathogens. Defensins possess similar traits to that of cathelicidins; they are antimicrobial, synthesized as an inactive peptide and they can regulate host immunity. α-defensins mainly protect mucosal surfaces from pathogens and they are capable of up-regulating TNF-α synthesis, and thus inducing inflammation. In addition to being antimicrobial, β-defensins are chemoattractants for immune cells. Moreover, they can induce the release of various mediators such as histamine and prostaglandin D2 and can modulate host defenses by activating certain receptors [145].

The previously mentioned study by Kaus *et al*. showed that human β-defensin 2 (hBD-2) and 3 (hBD-3) expressions in burn wounds drastically increased and suggested inflammatory stimuli and bacterial colonization as possible reasons for the increase [126].

hBD-1 and hBD-2 are primarly antimicrobial against Gram(−) bacteria while hBD-3 has antifungal activity as well as broad-spectrum antibacterial activity [145]. In another study it was reported that MDR bacteria were susceptible to β-defensin 4 [146].

Defensins have been patented for pharmaceutical use in various applications. Human neutrophil peptide-1, an α-defensin, was patented by Bevec *et al*. [147] and was described as effective for prophylactic and therapeutic treatment of many diseases. Novel human and mouse β-defensin genes and peptides were identified by Casavant *et al.* [148] who acquired the patent for them. Compositions containing the novel peptides could be used therapeutically through a multitude of administration routes including topical treatment of burn wounds. Jia *et al.* [149] patented hBD-3 and stated that it could be used in tandem or simultaneously with other antimicrobials. Beuerman *et al*. [150]altered the amino acid sequence of hBD-3 by substituting one or more cysteine residues with different amino acids which produced a less cytotoxic variant of the molecule compared to the wild type.

Compounds that stimulate the release of defensins have also been patented. Waite *et al.* [151] discovered that essential fatty acid triglycerides such as α-linoleic, palmitic, oleic and stearic acid triglycerides extracted from the seeds of certain plants from Echium and Linum genera induced the secretion of β-defensins who then formulated compositions with suitable carriers for dermatologic use.

Similarly to hCAP-18, bioengineered NIKS expressing hBD-3 were also tested in murine burn models and was shown to inhibit *S. aureus* growth in tested wounds. The study also showed that tissue transplants comprising the engineered cell lines were more effective compared to topical administration of synthetic hBD-3 [152]. The previously mentioned patent by Centanni *et al.* also includes genetically modified NIKS that can express defensin peptides [142].

7.3. Lactoferrin

Lactoferrin is an iron-binding protein belonging to the transferrin family; it was first isolated from bovine milk. The peptide mainly functions as an iron carrier in milk but is also found in neutrophil granules and bodily secretions such as tears, saliva, sweat, nasal and genital secretions as a part of body’s innate immune system. Lactoferrin exhibits antibacterial and antifungal effects by sequestering iron inside the medium thus depriving the organisms of the essential metal. Additionally, the antiviral effects of lactoferrin have been attributed to its ability to bind to certain glycosaminoglycans found on cell membranes which might prevent some virions from entering the host cells [153]. Lactoferrin may have some immunomodulatory effects as well. In addition to binding iron, lactoferrin molecules were also found to bind endotoxins, which might prevent endotoxins from triggering immune responses and causing septic shock [154].

Lactoferrin peptides have been patented for topical use by Riccio [155]. Many lactoferrin variant peptides have also been patented for their improved antimicrobial and anti-inflammatory effects [156]. Lactoferrin has also been combined with lysozyme, another endogenous AMP, in a novel topical formulation by Deeter *et al.* [157] which can be applied to the skin for treating a multitude of diseases or disorders including burn infections.

In a study by Tang *et al.* a recombinant human lactoferrin (rhLF) promoted keratinocyte proliferation, migration and wound healing [158]. Porcine dorsal burns that were treated with hydrogels containing rhLF achieved significantly higher percentages of epithelial regeneration compared to control groups. Moreover, rhLF acted as a chemoattractant for immune cells and facilitated release of immune mediators which accelerated wound closure. Novel rhLFs have been patented; a lactoferrin variant that was modified at the N-terminal by Engelmayer *et al.* [159] performed significantly better in treating wounds than recombinant human platelet-derived growth factor.

Lactoferrin derivatives, lactoferricin and lactoferrampin, are truncated forms of the peptide which are cleaved from the N-terminal of bovine lactoferrin. These peptides exhibited a more potent antimicrobial activity than the uncleaved lactoferrin. Both lactoferricin and lactoferrampin may cause membrane disintegration due to their cationic charge [160, 161]. A study by Haney *et al*. [161] concluded that a chimeric combination of these 2 peptides, which was initially synthesized and patented by Bolscher *et al*. [162], achieved greater membrane degradation of *E. coli* compared to non-chimeric forms administered separately or in a mixture.

8. METAL BASED ANTIMICROBIALS

Metals have been used for their antimicrobial properties for millennia despite their mechanisms were not known. Today, many metal containing preparations are established pharmaceuticals with known mechanisms and used in treating various diseases and infections [163].

8.1. Silver

Silver products have been used in burn care for over 200 years [164]. Silver in its ionized form, Ag^+ is known to react with thiol functional groups, thus inhibiting many vital enzymes of bacteria [165, 166] (Fig. (7). Moreover, silver prevents cellular division by accumulating in intracellular vacuoles and causes membrane damage by adhering to the plasma membrane and disrupting the natural electrical potential due to its high conductivity [167, 168]. Although many silver preparations are widely accepted and used in burn care, and in wound care in general, researchers continue to work on developing more efficient derivatives or delivery systems of silver for a better overall curative effect.

Silver molecules, metal or salts, can be incorporated into gels, creams and ointments for topical applications and exhibit biocidal effects at the wound site. These preparations may also include other antimicrobials or wound healing products in a mixture or in a complex with the silver metal.

8.1.1. Colloidal Silver Preparations—Traditionally colloidal silver solutions were used in clinical practice, but silver salts then replaced colloidal silver solutions due to their improved formulation which allows for enhanced stability, easier ionization capability and decreased formation of precipitates [13].

Despite certain disadvantages, colloidal silver preparations can be used for treating acute and chronic cutaneous lesions and burns. Gennari *et al.* [169] produced a composition containing silver in colloidal metal form and hyaluronic acid (HA) in the form of sodium hyaluronate between 130 and 230 kDa. The specific interval of HA acts as a potentiator of the antimicrobial activity of silver colloids. In addition to promoting fibroblast proliferation, HA also reduces the toxic effects of silver on fibroblasts, decreasing the possibility of delays in wound healing due to silver toxicity.

8.1.2. Silver Nitrate—Silver nitrate (SN) is one of the most commonly used silver preparations (the other being silver sulfadiazine) for burn wound infections. The salt can be combined with many other compounds to provide a better treatment unless there is pharmaceutical incompatibility. Hadar *et al.* [170] included menthol; a non-antimicrobial, local anesthetic substance known for its cooling effects which may relieve pain associated with burns, in a SN composition and observed synergism between said compounds. This synergism refers to an observed antimicrobial effect of the combination greater than the individual effects of the compounds. The synergism allowed silver ions to be antimicrobial at lower concentrations thus preventing possible irritation, discoloration and/or delays in wound healing and epithelization, which may occur at higher doses. The composition also contained a hyperosmolarity inducing agent such as polyethylene glycol (PG) or glycerol suggesting these molecules may protect the wound site from exudates and allow for better healing in general.

A patent was issued by Katzner *et al.* [171] for a composition comprising of SN and β-glucan, an immunomodulatory agent that can activate the immune system [172]. An *in vitro* study done with said composition tested for zones of growth inhibition in agar plates of various bacteria cultures. Wound dressings or lotions were put in the plates and the diameters of the zones were measured. Although β-glucan was expected to induce bacterial growth, the compound did not hinder the bactericidal activity of the silver preparation. Thus the composition may exhibit antimicrobial and immunostimulating properties *in vivo*.

SN and 4,5-diazafluorenone, a picolinaldehyde compound, were complexed by Langer *et al.* [173]. The complexed material exhibited antibacterial activity at lower concentrations compared to conventional antibiotics against several pathogens. These compounds were tested using broth microdilution method on many common burn wound pathogens such as *P. aeruginosa*, *K. pneumoniae* and *S. aureus*. In addition to the antibacterial effects, the compound also inhibited the growth of *C. albicans*.

SN has been patented as an antibiotic adjuvant by Collins *et al.* [174]. The method incorporates SN into the treatment regimen at concentrations at which the silver product is not bactericidal on its own but allows the composition to achieve a broader spectrum and potentiates antimicrobial efficacy. Sublethal SN contributes by disrupting the iron homeostasis and assisting in the production of reactive oxygen species, which are known for their antimicrobial properties.

8.1.3. Silver Sulfadiazine—Silver sulfadiazine (SSD) is a topical antimicrobial compound, composed of a sulfonamide and silver. Although there is concern about

emerging resistance and possible side effects, SSD is widely accepted as the first drug of choice for burn wound infections. As various side effects of SSD are identified, researchers have started making compositions that would prevent these undesirable effects from occurring. A study by Lee *et al.* [175] suggested epidermal growth factor (EGF) may be added into a cream or a gel containing SSD in order to prevent tissue regeneration delays. Vadrevu *et al.* [176] patented a composition that contains recombinant human EGF with SSD. A preparation consisting of SSD and chitosan was patented by Vanangamudi *et al*. [177]. Chitosan is known for its antimicrobial and wound healing properties [178] thus a composition of SSD and chitosan may have significantly more therapeutic effects on burn wounds than preparations with SSD alone.

Delivery of SSD is also an important factor in maximizing the antimicrobial effect. Two 1% topical SSD formulations with different delivery methods were compared in a clinical trial. Powdered spray form of SSD as opposed to cream form achieved greater antimicrobial efficacy; 80% prevention of infection as opposed to 77% with cream, 49% sterilization compared to 34% with topical cream on infected skin. Labruzzo, who had also conducted the clinical trial, patented the SSD composition which uses powdered spray delivery method [179].

8.1.4. Nanosilver Preparations—Silver cations due to their positively charged nature may bind to proteins and other anions on the wound site such as chloride which precipitates silver chloride [13]. The binding process prevents silver ions from penetrating inside the wound bed thus hindering its antimicrobial potency [180]. Production of nanoscale SSD particles may allow the drug to penetrate deeper thus achieving greater antimicrobial effects. Arora *et al.* [181] patented nanonized SSD creams of various concentrations and compared said creams to micronized 1% SSD topical cream in terms of antimicrobial efficacy against *P. aeruginosa* infections in mouse superficial burn models. Nanoparticle formulations containing 1% SSD achieved greater log reduction of bacterial burden than the micronized formulation of the same concentration.

Silver nanoparticles may be delivered to the wound site by other nano-sized carriers such as silica nanoparticles, a method patented by Santra [182]. Another patent was acquired by Axcelon Biopolymers Corporation [183] where silver nanoparticles are attached to bacterial cellulose fibers to manufacture antimicrobial wound dressings. Holladay *et al.* [184] developed a sprayable hydrogel containing silver nanoparticles which may be used as burn wound dressings. Mousa *et al.* [185] invented silver nanoparticles conjugated with various other beneficial molecules such as hyaluronan or heparin to achieve multiple therapeutic effects with one molecule. Hyaluronan was previously described as a wound-healing agent. Heparin is known for its anticoagulant, pro-angiogenic and wound healing properties [186].

8.2. Bismuth

Bismuth is a metalloid element that has several medicinal applications when combined with other functional groups or molecules. Historically, bismuth subsalicylates have been used in gastroenterology for antidiarrheal and antiacid purposes [187]. Lately other bismuth compounds were studied as possible therapeutic agents [188].

Bismuth was combined with certain thiol chelators to form compounds such as bismuth-1,2-ethanedithiol and bismuth dimercaprol. The combination greatly augmented the intrinsic antimicrobial effects of the metal and its solubility [189]. These compounds exhibit their antimicrobial effects both *in vivo* and *in vitro* [190, 191]. Bismuth-thiols in sub-lethal doses inhibit polysaccharide capsule production in bacteria, severely hindering their pathogenesis [192]. The inhibition of capsule synthesis allows bismuth compounds to disrupt biofilm formation and have biofilm dispersing capabilities. Additionally, bismuth may interfere with the iron metabolism of bacteria. Baker [193] patented micronized bismuth-thiol compounds that are topically applicable to wounds. Bismuth-thiols of the patent were also found to show synergism with certain antibiotics.

Bates *et al*. [194] invented a matrix that could be remodeled *in vivo* made of small intestinal submucosa extract comprising bismuth-thiol compounds. The invention is claimed to be beneficial for treating biofilms formed on wounds because of the biocidal bismuth compounds. Also, the novel product promotes wound healing by supplying a rapidly vascularized matrix on to the lesion.

8.3. Copper

Copper (Cu) is a metal with intrinsic antimicrobial properties [195]. Cu was used to sterilize water and to treat various wound infections even before the discovery of microbes [196]. In a study by Karpanen *et al*. [197] a variety of objects and materials that contained copper in them, such as frequently touched surfaces like toilet hardware were tested for their ability to prevent transmissible diseases in hospitals and proved to be substantially effective in reducing nosocomial pathogens. The antimicrobial mechanism of Cu may involve redox reactions and formation of radicals [198] or binding to certain functional groups and inhibiting various enzymes [199]. Fitzgerald *et al.* [200] formulated compositions with a copper salt and a quinone compound, as an adjuvant for stabilizing the salt, which are therapeutically effective against *Staphylococcus* spp. and may be used on infected skin lesions such as burn wounds.

8.4. Gallium

Gallium (Ga) is a metallic element which is not readily found in nature in its pure form. However Gallium (III) compounds can are observed in ores of several other metals such as zinc and aluminum [201]. Ga^{3+} cations have been studied as possible antimicrobials. The cationic structure resembles ferric iron (Fe^{3+}) in terms of ionic radius. Fe^{3+} is an important trace element in eukaryotic biological systems, bacterial iron homeostasis and metabolic pathways. Due to its resemblance to Fe^{3+} and its inability to be reduced, the cation acts as a competitive inhibitor of Fe^{3+}in many essential redox reactions and cell proliferation enzymes [202]. DeLeon *et al.* [202] showed the antimicrobial effects of gallium (III) maltolate salts in *P. aeruginosa* infections of murine burn wounds. Britigan *et al.* [203] patented a composition containing gallium that is applicable for the inhibition of biofilm formation on burn wounds. The structural weakening of a biofilm causes bacteria to be susceptible to immune agents and antimicrobials that would otherwise be ineffective.

9. HALOGEN BASED ANTIMICROBIALS

Of the four common halogen elements (fluorine, chlorine, bromine and iodine) two (chlorine and iodine) have been commonly employed as antimicrobials.

9.1. Chlorine

Chlorine and its derivatives were commonly used for disinfection and sanitization purposes since the 19th century [204]. Previous research indicates that compounds containing chlorine such as sodium hypochlorite exhibit their antimicrobial effects primarily by forming hypochlorous acid (HOCl) [167]. Deleterious effects of HOCl associated chlorine substances are thought to be on the DNAs [205] and oxidative phosphorylation mechanisms [206] of bacteria. Other studies have shown additional antimicrobial effects on bacteria [207], suggesting the mechanism of action of these compounds should be analyzed further. HOCl also possesses virucidal activity and have been shown to damage the nucleic acids [208], and capsids of virus [209].

In recent years many patents applications were filed for antimicrobials comprising chlorine. Dibello *et al.* formulated [210] a stabilized HOCl composition that can be used for disinfecting contaminated burn wounds. Rodewald *et al.* [211] invented a hydrophobic wound dressing that contains HOCl. Said wound dressing deactivates pathogens and disrupts biofilms on the wound site by releasing HOCl and binds to the deactivated microorganisms thus cleansing the wound when the dressing is removed. A chlorine (II) dioxide composition suitable for burn wounds was formulated by Castellana *et al.* [212] which showed significant (more than 5 log) reduction in MRSA and *P. aeruginosa* numbers in an *in vitro* assay on bacterial biofilms.

9.2. Iodine

Molecular iodine has broad-spectrum antimicrobial properties and has been used as an antiseptic for some 150 years. The complete mechanism of action is unknown however; molecular iodine is believed to bind sulfur groups in enzymes and possibly other functional groups in essential macromolecules and deactivate them to exert antimicrobial activity. Due to disadvantages such as poor solubility, staining, toxicity and irritant properties iodine solutions were replaced by iodophors [213] despite regular iodine having greater antimicrobial capabilities against fungi and spores. Iodophors (the most common being povidone-iodine (PVPI)) are compounds that contain molecular iodine combined with a solubilizing agent that allow sustained release of molecular iodine; they may be used both as antiseptics and surface disinfectants [167, 214]. Although numerous patents have been issued for iodine preparations that can be applied to burn wounds, their efficacy has been disputed. In a study that tested various, well established drugs in burn wound treatments such as SSD, SN, chlorhexidine and a PVPI ointment for their *in vitro* antimicrobial potency against bacteria isolated from infected burn wounds, the PVPI ointment failed to show any antimicrobial activity against the isolates [215]. Nevertheless, compositions containing iodine listed on Table 6 have been patented and have been claimed as suitable for treating burn wounds.

10. ANTIMICROBIAL COMPOUNDS

The difference between an antibiotic and an antimicrobial compound is subtle but real. Antibiotics inhibit some important biological process necessary for microbial proliferation or survival, and these processes are usually based on enzymes or protein or DNA synthesis. Antimicrobial compounds interact with microbial cells in less specific (but no less deadly) ways and a very common motif is membrane disruption often due to pronounced cationic charge.

10.1. Chitosan

Chitosan is isolated from crustacean shells and is a biopolymer made up of randomly acetylated and de-acetylated D-glucosamine units that can be used for many wound applications. The amino sugar monomers are of cationic nature and retain their positive charges in polymer form. The positively charged structure of chitosan is the base for its biotherapeutic properties such as; antimicrobial efficacy, fast blood coagulation and electrostatic immobilization of wound pathogens. Additionally, chitosan has some wound healing properties such as promoting and regulating reepithelization [216].

A novel tannin-chitosan composite was invented by Reed *et al.* [217] which is claimed to be superior to preparations solely made up of chitosan. Tannins are polyphenolic, negatively charged compounds known for their antimicrobial effects on viruses [218], bacteria [219] and parasites [220]. Tannins are further discussed under “Natural Products” in this article. Tannin and chitosan molecules bind together electrostatically to form the composite. Together, chitosan and tannin exhibit synergism as tannins bestow additional bacteriostatic and fungistatic effects on the composition as well as decrease the rate of biodegradation of chitosan nanoparticles, improving sustained release. The composite can be applied topically as a hydrogel, liposomal coatings and/or nanoparticles to wounds to decrease the microbial load and promote wound healing. Moreover the composition can comprise further phamaceuticals or other antimicrobials as it encapsulates said products and function as a controlled release system.

Montenegro *et al.* [221] patented a chitosan composition for use as an antimicrobial and epithelial cell proliferative compound directly applicable to wounds for therapeutic effects. Gregory *et al.* [222] patented a wound dressing comprising chitosan, which functions as an antimicrobial barrier and a hemostatic patch. Apex Laboratories Private Limited acquired a patent [223] for a cream containing chitosan and framycetin sulphate, a topical aminoglycoside antibiotic, that is claimed to provide superior overall wound care in terms of treating infections and healing effects than existing preparations.

10.3. Ceragenins

Ceragenins, (Fig. 8F) also called cationic selective antimicrobials (CSAs), are synthetic steroidal drugs derived from cholic acid, a major bile acid [224]. They were invented and then patented by Li *et al.* [225] as a possible new method for treating infections. Similarly to antimicrobial peptides (AMPs), they are cationic and affect the outer membrane of bacteria. CSAs exert their membrane disrupting (depolarizing and permeabilizing) effects on both

Gram(+) and Gram() bacteria but the susceptible spectrum varies between different species of ceragenins, CSA-13 being the most potent [226]. Ceragenins may be administered as topical therapeutic agents to infected burn wounds, as indicated in the patent description.

Despite their mechanism of action, which resembles that of AMPs, these molecules are immune to the main resistance mechanism against AMPs: namely proteolysis, due to their steroidal structure. However, bacteria are capable of altering their lipid compositions in their cell membrane, which is the foundation for tolerance development to CSAs in bacteria. Certain lipid compositions of bacterial cell membranes comprising high amounts of uncharged lipids and phosphatidyle-thanolamine molecules were studied for the amount of depolarization in the membrane upon CSA treatment. Although the depolarization was limited, the treatment still proved to be bactericidal [227].

10.4. XF Porphyrins

XF porphyrins represent a new class of pharmaceutical that was developed and patented by Love *et al.* [228] that have a dicationic porphyrin skeleton. Although originally developed to be photosensitizers for photodynamic therapy, they were subsequently found to be active without light excitation. Research suggests that the mechanism of action is via bacterial membrane disruption. *In vitro* studies of XF-73 has shown its antibacterial efficiency against many Gram(+) and Gram(−) strains, Gram(−) activity was relatively less compared to Gram(+), including many common burn wound pathogens, irrespective of their innate or acquired resistances [229]. XF-70 has also displayed potent and similar bactericidal effects *in vitro*. Additionally, both compounds were effective against bacterial biofilms [230].

Due to their unconventional structure, XF drugs are claimed to exert strong antimicrobial activity without the development of resistance. A study conducted by the inventors showed that there was no significant increase in the MIC value of XF-73 despite continuous usage at sub-inhibitory concentrations whereas MIC values of conventional antibiotics such as fusidic acid, mupirocin and retapamulin significantly increased over passages and the emergence of resistant *S.aureus* strains was evident [231].

A study by Love *et al.* tested XF-70 on *S. aureus* infected murine burn wound models and compared its therapeutic effects to that of an SSD cream and silver impregnated wound dressing. It was reported that there was no significant difference in reducing inoculated bacteria between the treatment groups but all topical treatments reduced the bacterial load on the wound significantly compared to the saline treated control groups [232].

In addition to their inherent antimicrobial properties, XF class drugs may be used as photosensitizers due to their porphyrin skeleton and have been patented for photodynamic therapy (PDT) applications as well [233]. The reactive oxygen species produced by the photodynamic inactivation of these drugs possess antimicrobial traits and are effective against a broader spectrum of pathogens [234]. It has also been demonstrated that the compound exhibits fungicidal activity against *C. albicans* when used as a photosensitizer with blue light excitation [235].

10.5. Chlorhexidine

Chlorhexidine (CH, Fig. 3E) is a common antiseptic agent that is used in many oral hygiene [236] and hand sanitization products [237]. The substance possesses broad-spectrum activity and causes low irritability. CH at sufficient biocidal concentrations exhibits antibacterial and antifungal effects by damaging the cytoplasmic membrane thus causing cellular leakage and disrupting the membrane potential [167, 238]. Conversely, the compound has been shown to coagulate intracellular constituents at higher concentrations [239]. Despite its broad bactericidal and fungicidal effects, various studies have shown that CH has limited antiviral properties [240, 241]

In a study CH was of therapeutic use for reducing MRSA in rat burn wounds [56] while in another study it failed to provide significant reduction of *C albicans* in similar lesions [242]. A clinical research reported that CH was able to show antimicrobial activity against bacteria isolated from infected wounds of burn patients [215]. A device that contains CH was patented by Rucinski [243] for therapeutic wound irrigation and wound infection prophylaxis.

11. NATURAL PRODUCTS

Natural products have always been the basis for many if not most pharmaceutical products but they have been largely cast aside with the recent emergence of synthetic drug era [244]. However, research indicates that a vast majority of the drugs developed from 1980–2010 were based on natural products [245]. Abandoning the search for new natural substances and relying on synthetic compounds may have caused a great loss of potentially useful medicines particularly regarding novel anti-infectives against the increasing number of antibiotic resistant microbes.

Various natural molecules possessing antimicrobial traits and information about their patented compositions are given in Table 7. The antimicrobial structures contained by some of the listed botanical extracts are shown in (Figs. 8D and 8E).

11.1. Tannins

Tannins are polyphenolic compounds that can be extracted from many plant species [246–248] and can be found in many common food items such as tea [249], red fruits and vegetables and cocoa [250]. They are either found in their non-hydrolyzeable, acid form or hydrolyzeable ester forms.

Plants containing tannins have been known for their antimicrobial properties and have been used in folk medicine [251]. Current scientific research has shown that tannins have both bacteriostatic and bacteriocidal effects despite relatively higher MIC values compared to conventional antibiotics. Tannic acid, a hydrolyzable tannin which is the ester of gallic acid and glucose, has been found to be the most potent among these natural compounds [252]. Tannins are thought to exert antimicrobial potency through inactivating enzymes or substrates by forming complexes with them [253]. It has also been suggested that the iron binding properties of tannins may play a role in their antimicrobial mechanisms [254]. A study by Akiyama *et al.* [253] reported that tannin compounds could inhibit the coagulase

activity of *S. aureus* at varying MIC values. Moreover, tannins drastically improved the antimicrobial efficacies, and reduced the MIC values, of several antibiotics that were tested against *S. aureus.* In addition to their antimicrobial properties tannins have anti-inflammatory and wound healing effects, a highly therapeutic combination that can be used in healing burn wound infections [255].

Although these compounds have been known and used to treat burn wounds for some time, their use was abandoned due to some data suggesting hepatotoxic side-effects. However, the scientific basis of hepatoxicity claims are currently disputed as the dosage of tannin treatments were not properly adjusted to exert selective-toxicity towards pathogens in some studies [255]. Recent studies and reviews have suggested tannins or plant extracts containing tannins could be reinstated as topical treatment regimens for wound and burn infections [256, 257]. Several recent patents have been issued for pharmaceutical compositions comprising tannins that are applicable to cutaneous lesions [258–260].

11.2. Essential Oils

Essential oils (EOs) are volatile substances extracted from various plants and they have myriads of applications such as in cosmetics, culinary and medicine. Many studies have reported that certain EOs possess antimicrobial properties and their use has been suggested in topical management of wound infections [261, 262].

An *in vitro* study by Carson *et al*. [263] has shown that *S. aureus*, including MRSA strains, are highly susceptible to tea tree oil (TTO). TTO extract also achieved promising results in terms of healing burn wounds when it was administered topically to porcine burn wounds [264]. Valencia orange oil (CPV) is another EO that has shown antimicrobial efficacy against *S. aureus in vitro.* Additionally, CPV did not exhibit any toxicity to tested keratinocyte cell lines [265]. Several other medicinal EOs are shown in Table 7.

12. REACTIVE OXYGEN SPECIES (ROS) GENERATORS

ROS are highly reactive molecules containing oxygen such as hydroxyl radical, hydrogen peroxide, HOCl and singlet oxygen. These molecules have the tendency to react with biological macromolecules and cause cellular damage [266]. Organisms are able to tolerate ROS with their antioxidant enzymes including superoxide dismutase and catalase. The imbalance between the acitivity of the antioxidant agents (enzymes and quenchers) and ROS creates oxidative stress [267]. Considering the damaging effects of ROS, they have bactericidal and virucidal activity which human cells benefit from while combatting pathogens [268]. Thus, in medicine, ROS supplying drugs and systems have been developed for pharmaceutical use in order to fight infections.

Norton *et al.* [269] invented a method and apparatus for producing an electrolyzed saline solution (ESS) that supplies regulated amounts of relatively stable ROS to the site of infection. The invention advances the technique by applying the ESS solution topically and covering several possible areas of therapeutic use including the treatment of burn wound infections. Regarding its preparation, an effective voltage is applied between the cathode and the anode which are placed in the saline solution, so that a balanced mixture of ROS

appear. ESS containing regulated amounts of ROS ensures minimal toxicity, thus a safe way of treatment that also enhances the immune system by increasing its ability to detect and destroy malfunctioning cells by an undisclosed mechanism of action.

Other inventions supplying ROS which are produced by lactoperoxidase (LP) system were patented by McCay *et al*. [270] and by Galley *et al*. [271]. The body generates ROS via microbicidal peroxidase systems such as LP and myeloperoxidase as a part of the innate immune system [272]. As shown in (Fig. (9), the substrates of LP enzyme include anions like thiocyanate, bromide, iodide and molecules such as hydrogen peroxide which all get oxidized during the reactions catalyzed by LP. LP's biostatic effect has a broad spectrum consisting of catalase positive Gram(−) and Gram(+) bacteria, viruses and fungi such as C. albicans that has been detected to be susceptible to hypoiodite, an end product of an LP catalysed redox reaction [273]. The bacteriostatic effect mainly results from the oxidation of thiocyanate to hypothiocyanite (HOSCN) and anions to hypohalous acids in presence of hydrogen peroxide [273–277]. After crossing the bacterial cell wall, HOSCN reacts selectively with the sulfydryl groups of bacterial proteins [275, 278], particularly enzymes of the glycolytic pathway, thus inhibits bacterial growth but is better tolerated by human cells. In the human body, LP system functions mainly in human airways and mammary glands, as well as mucosal secretions [279]. In medicine, LP has eventually found application as an effective antimicrobial agent in the treatment of burn wound infections.

The invention of McCay *et al* [270] describes various microbicidal compositions containing either iodide/thiocyanate ions which react with hydrogen peroxide or similarly with peroxide-releasing-sodium percarbonate, or chloride ions which react with glucose and glucose oxidase. The two reactions require the presence of lactoperoxidase and chloroperoxidase, respectively. Before both types of compositions are applied to the infection site, they are treated with catalase in order to remove excess hydrogen peroxide that might cause oxidative stress to human cells when applied. Optionally, catalase can also be administered to the infection site. In addition, controlled amounts of hydrogen peroxide strengthens the antimicrobial efficacy of compositions by its toxicity to microorganisms and immune stimulating properties. Regarding the composition containing glucose and glucose oxidase rather than hydrogen peroxide, in this case, the source of peroxide is the chemical reaction that occurs between glucose and glucose oxidase resulting in a peroxide and an acid.

As the patent application by McCay *et al*. [270] claims, all the prior art and patents have demonstrated the bacteriostatic activity of LP-related treatments, whereas his method notably demonstrates bactericidal potency against some clinically important burn wound pathogens such as *E. coli*, *S. aureus*, *P. aeruginosa* and streptococci, including their resistant strains. The trials for the compositions showed that the bactericidal action of the said method is exclusively due to the action of the ROS with no residual hydrogen peroxide and no other synergistic antimicrobial agents. Most importantly, the compositions are applicable to burn wounds in the form of poultice and effective against biofilms.

The invention by Galley *et al.* [271] discloses compositions comprising iodide and thiocyanate ions together with glucose and glucose oxidase. The compositions may additionally include antioxidants and peroxide-generators such as lactoperoxidase.

13. NITRIC OXIDE GENERATORS

Due to its numerous important functions and versatility, nitric oxide (NO) was named “The Molecule of the Year” in 1992 by Koshland, the editor of Science at that time [280]. Furchgott, Ignarro and Murad went on to win the Nobel Prize in Physiology or Medicine in 1998 for describing the effects of NO as an endogenous signaling molecule. NO is a highly reactive free radical that has a low half-life which is synthesized from L-arginine, by nitric oxide synthase enzymes in mammalian cells both constitutively and upon induction by stimuli such as infection or inflammation [281]. NO has many known functions such as: neurotransmitter, vasodilator, immunomodulator, anti-inflammatory and a broad spectrum antimicrobial agent. Moreover, NO regulates platelet aggregation and may have a role in protecting tissues from ischemic stress caused by deposition of immune complexes [282]. Its intrinsic antimicrobial activity may be attributed to a variety of mechanisms of action: chemical alterations of DNA and DNA repair enzymes, and lipid damage by acting as reactive nitrogen oxide species [283].

13.1. Gaseous Nitric Oxide

Because the antimicrobial activity of NO can affect a plethora of pathogens, novel topical treatments incorporating NO have emerged. In a study by Ghaffari *et al.* [284] exogenous NO in gaseous state was directly administered to puncture wounds without any carriers in which the treatment achieved significant reduction in inoculated *S. aureus* numbers. It was also reported that gaseous NO (gNO) treatment did not cause any cytotoxic effects to skin cells tested such as fibroblasts and keratinocytes and thus did not hinder the wound healing process [284]. A topical formulation comprising gNO carriers has been patented by Av-Gay *et al.* [285] which are capable of releasing therapeutically effective amounts of the gas when applied to a wound. Devices capable of releasing gNO have also been patented by Prakash *et al.* [286] where said device provides a storage environment deprived of oxygen for gNO to prevent the compound from being oxidized [282].

13.2. Nitric Oxide Releasing Nanoparticles

NO nanoparticles (NO-np) may be used to improve the potential of NO as an antimicrobial by allowing for controlled and sustained release of the molecule in a stable manner. Additionally, nanoparticles may help reduce the varying cytotoxic effects of NO on mammalian cells [287]. In a study by Mihu *et al.* [288] NO releasing nanoparticles were used as a possible therapy for *A. baumannii* infected wounds. The NO-np drastically lowered the bacterial load while also accelerating wound closure. The beneficial effects of NO-np were further demonstrated in another study by Martinez *et al*. [289] where a chitosan matrix comprising NO-np were used against *S. aureus* skin infections; both chitosan and NO possess antimicrobial and wound healing properties thus the composition exhibited synergism regarding both effects. The composition was patented by Friedman *et al.* [290] whom also patented another NO-np composition [291] that contained exogenous

gluthathione which is claimed to enhance the efficacy of NO-np. Yet another study by Macherla *et al.* [292] showed *in vivo* fungicidal activity of NO-np against *C. albicans* in murine burn wounds. Similarly to nanoparticles, synthetic macromers and oligomers that are capable of providing sustained release of NO and/or other drugs have been patented by Bezwada *et al.* [293] for therapeutic use.

13.3. Nitric Oxide for Wound Healing

Separate studies have also been done exclusively studying the wound healing effects of NO. An increased synthesis of NO was observed in healing wounds [294] whereas in chronic, non-healing wounds the synthesis of NO was suppressed [295], which suggests that NO has a role in modulating tissue repair. Pulfer *et al.* [296] developed a polyethylenemine cellulose NONOate (diazeniumdiolate) NO-releasing polymer which showed significantly higher percentages of healed wounds compared to control group when the composition was applied topically [297].

Various wound dressings and controlled release apparatus comprising NO releasing polysiloxane macromolecules [298] and S-nitrosothiol compounds [299] have been patented for wound treatment purposes. Many topical gels that contain NO-releasing molecules have also been formulated for topical use [300, 301]. A novel composition patented by Schoenfisch *et al.* [302] incorporates the use of a silver-based therapeutic agent with an NO-releasing macromolecular scaffold to achieve antimicrobial synergism and enhance the efficacy of silver inside the composition.

14. PHOTOTHERAPY

Phototherapy refers to the process of using light, both visible and/or non-visible, to treat a vast number of disorders in a minimally invasive manner. Phototherapy encompasses four distinct treatment regimens: photodynamic therapy (PDT), ultraviolet irradiation, blue light therapy and low-level laser (or light) therapy (LLLT). Although all have their own separate applications, their effects can be complimentary, particularly in burn wound treatments [303].

14.1. Photodynamic Therapy

PDT incorporates the use of photosensitizers (non-toxic dyes) and light at a specific wavelength together in a process called photoactivation to generate ROS when activated in the presence of oxygen. PDT has been mainly developed as a novel approach to malignancies and various dermatological disorders. However, due to the potent antimicrobial effects of ROS produced in the process, PDT has also been adapted as an infection treatment modality, photodynamic antimicrobial chemotherapy (PACT). Coupled with the rise in pathogens resistant to chemotherapeutic agents and a lack of useful antibiotics, the method proved useful in combating various disease causing microorganisms [304].

Photosensitizers (PS) may have many different structures but delocalized electrons in resonance and an overall cationic charge are common properties of these molecules when used for antimicrobial purposes [305]. Upon excitation by light, the PS transforms into a

highly reactive and relatively unstable excited singlet state. In its singlet state the molecule undergoes intersystem crossing, a light-independent process where the molecule changes its electron state, and transforms into its long-lived triplet state. The triplet state can survive long enough to react with molecular oxygen in its triplet state to form excited state singlet oxygen (a ROS) [13].

PDT exhibits antimicrobial effects both *in vivo* and *in vitro* [306]. PDT was reported as an effective treatment for burn wound infections by numerous papers. A study by Dai *et al.* [33] examined the effects of topical PDT on *A. baumannii* infected murine burns. The treatment provided substantial decreases in bacterial bioburden and was particulary effective when applied immediately after wound contamination. Similar studies have been conducted using different PSs and achieved comparable results regarding PDT's antimicrobial effects [307].

Many endogenous molecules such as porphyrins, and flavins can act as PSs [308]. A list of synthetic derivatives of these compounds and many other novel PSs with different structures that have been patented for pharmaceutical use in PDT is shown in Table 8.

14.2. Ultraviolet Irradiation

Ultraviolet irradiation (UVI) also known as ultraviolet germicidal irradiation (UVGI) uses ultraviolet-C (UVC), short-wavelength ultraviolet radiation (200–280 nm), to kill microorganisms. UVC damages the microbial nucleic acid by causing distortions and thus prevents replication and deactivates the pathogen. UVGI can be used for various applications such as sterilizing a medical device [309], cleaning the skin of a patient prior to surgery [310] or eradicating microbes on an infection site [311]. Although UV light is known to be detrimental to human cells upon prolonged exposure, research indicates that microorganisms are more susceptible to UV inactivation than human cells, which suggest UVGI may be a viable modality in treating infections [311].

Furthermore, UVC has been shown to possess certain wound healing properties and has been used by healthcare practitioners [312]. It has been suggested that UVC achieves its wound healing properties by inducing epidermal hyper-plasia and and increasing microcirculation on the site where it is applied [313].

In a study by Dai *et al.* [178] 3rd degree *C. albicans* infected mouse burn wound models were treated using UVC. The fungal bioburden significantly decreased and there was no visible side effect of the UV treatment to the animal. In a similar paper, Dai *et al.* [314] studied the effects of UVC prophylactic treatment in burn wounds for various pathogens such as *S. aureus*, *P. aeruginosa* and *A. baumannii.* Directly after the wounds were contaminated with pathogens the lesions were exposed to UVC. Infection was established in control groups whereas the number of inoculated bacteria significantly decreased in UVC treated mice. Despite the fact that the study reported some damage to mice skin cells the damage was largely repaired suggesting the treatment is applicable in similar lesions.

14.3. Blue-light Therapy

Blue light (405–470 nm), has been found to exhibit antimicrobial effects without the presence of added exogenous PSs. Moreover irradiation with blue light is selective towards pathogens and has been shown to cause minimal inactivation of human cells such as keratinocytes. Although exogenous PSs are not used with blue-light therapy, the mechanism of action is believed to be similar to PDT with the photoexcitation of endogenous porphyrins which subsequently act as PSs to form ROS [315]. Blue-light therapy is already an established modality in treating acne [316], peptic ulcer [317] and has been further studied for treating life-threatening infections such as skin and burn infections [318, 319].

Dai *et al.* [318] showed that blue-light therapy was beneficial for reducing *P. aeruginosa in vitro* and in murine burn wounds while no significant damage was observed on keratinocytes *in vitro* or to the mouse skin. In another study, Dai *et al.* [319] again showed that blue-light could eliminate MRSA from infected mouse skin abrasions but also reported that biofilm formation by bacteria on wounds could decrease the efficacy of the treatment. The wound healing effects of blue-light therapy have also been studied. Adamskaya *et al.* [320] observed that blue-light irradiation treatment decreased wound sizes in murine excisional wounds compared to control groups. The suggested mechanism of action was that blue-light induced the release of NO. Adversely, Soyer *et al.* [321] reported a decrease in vascular endothelial growth factor (VEGF) upon blue-light treatment which may be deleterious in wound healing because VEGF promotes angiogenesis.

14.4. Low-Level Light Therapy

LLLT is a non-thermal, non-antimicrobial phototherapy using either laser or non-coherent light. Clinical applications of LLLT include alopecia treatment [322] and pain management therapies [323]. Various studies displayed LLLT's anti-inflammatory and analgesic effects when applied for treating orthopedic injuries and degenerative diseases [324]. LLLT also proved to be beneficial in treating muscle tissue for augmented oxidative performance [325].

LLLT is being extensively studied regarding its wound healing properties, an area of application which would be of great therapeutic use in treating burn wounds and wounds in general. Studies have shown cellular proliferation and increased collagen production *in vitro* upon LLLT application to the cell culture mediums [326]. A study by Gupta *et al.* [327] displayed the *in vivo* proficiency of LLLT, employing a wavelength of 810-nm, in murine wounds; the therapy promoted cellular proliferation and re-epithelization thus accelerating wound closure. Lesions of the LLLT treated group using 810-nm wavelength were significantly smaller to that of controls or other treatment groups that employed different wavelengths. In another study by Usumez *et al.* [328] 980-nm LLLT treatment was found to be the most effective in inducing expression of growth factors thus facilitating wound healing.

14.5. Titanium Dioxide Photocatalysis

Titanium (IV) oxide, also known as titanium dioxide (TiO_2) participates in a process called photocatalysis, whereby under light excitation it reacts with water and creates ROS and other free radicals. TiO_2 acts as a photocatalyst for various wavelengths of light, including

both ultraviolet and visible light. The method has been incorporated for surface coatings where TiO_2 can facilitate the production of free radicals on various surfaces upon excitation and deactivate microorganisms that are found on said surfaces [329].

The aforementioned method has been of particular use in hospital environments for combating nosocomial infections as Chung *et al*. [329] displayed that TiO_2-coated surfaces had significantly lesser amounts of bacterial load on them compared to non-TiO_2-containing coatings and they were termed “self-cleaning”.

Taxt-Lamolle *et al.* [330] have shown that activities similar to TiO_2 photocatalysis could be achieved without any light activation but through chemical activation by hydrogen peroxide. Taxt-Lamolle patented a composition comprising hydrogen peroxide and TiO_2 nanoparticles where the combination was claimed to have broad-spectrum antimicrobial activity and anti-inflammatory properties and could treat wound infections.

15. PASSIVE IMMUNOTHERAPY

Monoclonal antibody therapeutics entered into medical world around 1980’s. At first, monoclonal antibodies were fully murine which had a problem of high immunogenicity and low efficacy due to the protein sequences being encoded by murine DNA. During 1980s the idea of reducing murine-derived sequences was widely studied so that the antibodies would suit humans better. In 1980s most of the therapeutic antibodies were either chimerized or humanized [331].

The first fully human monoclonal antibody was approved by FDA in 2002, whereafter their use has taken a major place in medical therapy [332]. Humanization or chimerization of the antibodies are possible in a number of ways such as *in vitro* optimization of the molecules and engineering transgenic mice expressing human monoclonal antibodies [333, 334]. Fully human antibodies produced by mice have been developed in 2000s with the integration of human antibody genes to the murine DNA and the inactivation of murine antibody loci through gene targeting [334, 335]. These mice are called XenoMouse®.

As an exemplary patent [336], anti-*P. aeruginosa* lipopolysaccharide antibodies derived from transgenic mice can be reviewed. The invention utilizes a transgenic mouse having a substantial portion of inserted genome producing human antibodies whereas it is rendered incapable of producing its own murine antibodies, so that in case of an antigenic challenge they produce fully human antibodies. The antibodies may be isolated from the mouse or extracted from the cultured hybridoma cells. In burn wound infections, the local delivery of antibodies that can function as antagonists of various specific components of burn wound pathogens has proven useful and effective in relevant studies [337]. In addition, most of the popular patented antibodies have been developed against *P. aeruginosa*, the species showing one of the highest incidence in burn wound infections.

15.1. Flagellin Binding Antibodies

A bacterial flagellum is composed of filaments formed by the globular protein, flagellin [338]. Flagellins are important virulence factors enabling the characteristic rapid movement

of flagellated bacteria and facilitating bacterial invasion. Passive immunization against the flagellum structure has demonstrated decreases in virulence and lethality [339]. Considering that administering specific antibodies binding to flagellum/flagellin antigens of the bacterium results in a lowered level of virulence, monoclonal antibodies are useful for combating burn wound infections.

Neville [340] invented improved antibodies targeting flagellin type A and/or type B of *P. aeruginosa*. The antibodies disclosed in the invention either specifically bind to flagella or flagellins and they might be fully human, humanized or chimerized form of a human antibody. Furthermore, they might be dual-specific meaning that they are able to bind both type A and type B flagellins. The antibodies were produced by various host cells such as hamster ovary cells, mouse myeloma cells and plant cells comprising the nucleic acid encoding human immunoglobulins that are encoded by vectors integrated into the host cell. The invention was tested for the activity of antibodies in creams or gels intended for the treatment of topical PA infections associated with burn wounds and reported positive results.

In a study, Barnea *et al*. [339] tested the effect of anti-flagellin type A monoclonal antibodies on PA infection in a mouse burn model and drew some important conclusions. Anti-flagella antibodies reduced bacterial motility resulting in the failure of the bacteria to disseminate systematically but did not affect their ability to replicate in the first 48 hours after infection and when used as a prophylactic, treatment or combined regimen it achieved enhanced rates of survival, The combined regimen (pre-infection and post-infection) even achieved a 96% survival rate which is equal to that of Imipenem therapy.

15.2. Anti-Quorum Sensing Antibodies

Homoserine lactones (HSL) and their derivatives (Fig. 8A, 8B, and 8C) are bacterial signaling or quorum sensing molecules that enable bacteria to regulate their virulence factors and transform into a virulent form via cell-to-cell communication [341]. Charlton *et al.* [342] developed a method of treating and monitoring most Gram(+) and Gram(−) bacterial infections, but primarily PA infections, by administering immunoglobulins having high affinity to HSL-derived signaling molecules. In addition to the reduction in the amount of extracellular signaling molecules, and therefore in bacterial virulence, the therapy facilitated the monitoring of the bacterial load and assessment of the progress of infection. The inventors claim that anti-HSL antibody treatment was superior compared to anti-PcrV antibody treatment because it includes more bacteria in its spectrum of activity. The antibodies disclosed in the invention might be monoclonal or polyclonal which should be obtained from an animal host stimulated by the injection of antigen. They are topically applicable for a burn wound infection.

15.3. PcrV and Psl Binding Antibodies

Psl is a galactose and mannose-rich exopolysaccharide which is essential for *P. aeruginosa* biofilm formation by being a component of matrix biofilm [343]. PcrV is the V antigen of type III secretion system (TTSS) of PA and it is a relatively conserved component of TTSS which is a virulence mechanism through which toxins are injected into host cells in order to

initiate the infection [344]. Both antigens are targeted by a number of inventions describing monoclonal antibodies that bind to them.

Digiandomenico *et al.* [345] patented a method of preventing and treating *P. aeruginosa* infection by an anti-*Pseudomonas* Psl binding molecule. As claimed, the antibody can inhibit the attachment of PA to epithelial cells and/or promote opsonophagocytic killing of *P. aeruginosa*. The disclosed compositions are applicable to burn wounds directly and have been tested in burn wound models by the inventors.

Regarding a similar therapy that was invented by Digiandomenico *et al.* [346] and patented by MedImmune Limited, anti-Pseudomonas Psl and PcrV binding molecules can be administered in combination or in the form of a bispecific molecule, both of which exhibit synergism compared to separate administrations of the individual antibodies. The antibodies of the said invention are structured according to the aim of ensuring their ability to bind a number of Pseudomonas species and serotypes. Again, the molecules can be applied directly to burn wounds and have been tested for burn wound models. As the results of the therapy, delivery of type III secretory system (shown in Fig. (10) toxins is blocked, opsonophagocytic killing of *P. aeruginosa* is enhanced and the attachment of PA to epithelial cells is inhibited.

A synergistic effect might be observed when the binding molecules are administered in combination with an antibiotic, either ciprofloxacin or meropenem or both, as disclosed in the invention [346]. According to a study by Song *et al.* [347], the combination therapy of a murine monoclonal antibody against PcrV and one of three antibiotics against PA infection including ciprofloxacin, demonstrated an enhanced bactericidal effect in murine models of *P. aeruginosa* lung infection. The results of the study might also relate to burn wound infections.

Burn patients are quite likely to get polymicrobial infections. A patent was issued for treatment of a polymicrobial infection involving *S. aureus* and *P. aeruginosa*, which have the highest incidence in burn wound infections (statistics in Table 2). Yarranton [348] patented a method of treating a wound having a staphylococcus infection and a “low-level” pathogenic PA infection at the same time, by administering anti-type III secretion system molecules such anti-PcrV antibodies in a therapeutically effective amount to reduce the SA load in the patient. “Low level” refers to a Pseudomonas level that would not be treated with antibiotics and less than the level of Staphylococcus warranting a treatment. Although the levels of PA and SA infection within a patient are commonly regarded as inversely proportional, administration of anti-PcrV decreases the load of Staphylococcus as well, contrary to expectations. In some embodiments, the invention discloses a concurrent antibiotic treatment for SA which might also be administered before or after the administration of anti-PcrV antibodies.

15.4. Anti-*Enterococcus* Antibodies

Throsby *et al.* [349] invented human antibodies specifically binding to enterococci, a common burn wound pathogen. The molecules are said to be able to kill a list of Gram(−) and Gram(+) bacteria in addition to enterococci. One or more fragments belonging to

enterococci might be the targets of the binding molecules. They can be administered in a mixture containing additional antimicrobial agents.

16. THERAPEUTIC MICROORGANISMS

Although the field of medicine is usually trying to combat microorganisms that cause infections, today's technology permits doctors and researchers to also utilize microorganisms for the benefit of patients. These methods have not become conventional yet but with the ever-expanding list of drug resistant pathogens, therapeutic microorganisms may very well become the main course of treatment in burn wound infections. Treatment via microorganisms has many advantages compared to treatment via antibiotics such as relatively low development of clinically significant resistance and the ability to be combined with other treatment regimens without the risk of adverse reactions.

16.1. Bacteriophages

Bacteriophages are viruses that target, infect and "devour" bacteria, but were virtually abandoned as a viable treatment for infections when antibiotics were discovered after World War II. However, new interest in the subject of antibacterial phages has developed due to their efficiency against MDR bacteria. Although phage resistance in bacteria has been reported [350], numerous strains of phages can be delivered in a mixture to prevent the bacteria from developing resistance. Moreover phages can be genetically altered in a laboratory condition to produce a different strain thus allowing it to continue infecting bacteria that have acquired resistance to the previous strain.

Bacteriophages primarly have two different reproductive cycles. A lytic reproductive cycle is when a bacteriophage multiplies in a host bacterial cell and lyses the cell while exiting through the membrane, whereas in a lysogenic reproductive cycle host bacteria stays intact and the phage DNA is integrated to the host genome (see Fig. (11). Bacteriophages must remain in a lytic reproductive cycle rather than lysogenic to be bactericidal.

Heo *et al*. [351] patented MPK6 lytic phage, a bacteriophage in the Caudovirales order, that has shown antibacterial efficacy in mouse peritonitis-sepsis model against P. aeruginosa. MPK6 phage is also a suitable pharmaceutical in P. aeruginosa infected burn wounds.

Garcia *et al*. [352] invented a multitude of bacteriophages and peptides produced by those phages, shown in Table 9, that exhibit lytic antibacterial effects on common burn wound pathogens. The phages have been topically tested on wound infections caused by *A. baumannii*, *K. pneumoniae*, *P. aeruginosa and* MRSA. The detailed phages can be administered in combination with each other in a "phage cocktail" to achieve therapeutic effects against different bacterial groups.

Leah [353] produced a composition of bacteriophages that are restricted to a lytic reproduction cycle by *in vivo* genetic manipulation. The patented method allows bacteriophages that would otherwise have lysogenic reproductive cycles to be converted into potent antimicrobials. Bacteriophages are exposed to site-directed mutagenesis or any other mutagenesis that alters the genetic sequence of the gene that determines the reproductive

cycle thus changing the nature of its cycle. This method of manipulation is claimed to be effective in producing obligate lytic phages of many bacteria that are burn wound pathogens such as but not limited to *K. pneumoniae, P. aeruginosa, A. baumannii* and *Enterobacter* spp. The composition can be prepared with the phages that are fixed on appropriate nanoparticles and applied topically.

Current biomedical technology allows bacteriophages to have multiple applications rather than just using them for killing bacteria. While treating infections, bacteriophages may also be used in a manner where they act as adjuvants to various antimicrobials or other phages. Collins *et al*. [354] engineered and produced lytic, including but not limited to M13, and lysogenic, including but not limited to T7, bacteriophages that potentiate antimicrobial efficacy by inhibiting innate or acquired antimicrobial resistance genes and/or suppressing SOS response genes such as lexA. These phages may be used while treating for common burn wound pathogens and have been tested with antibiotics from quinolone, aminoglycoside or β-lactam groups. Furthermore the engineered phages can be sequenced to express certain beneficial enzymes against burn wound infections such as biofilm-dispersing enzymes. Zegans *et al.* [355] also demonstrated that lysogenic phages, DMS3, can distrupt biofilm formation of *P. aeruginosa*.

R-type bacteriocins, a type of small AMP, resemble both morphologically and functionally tail-like structures of bacteriophages. Both these structures and bacteriocins act as antibacterials, they bind to virulence and stress factors or structural moieties. Scholl *et al.* [356] patented a P4 *P. aeruginosa* bacteriophage that has been genetically reengineered with a P2 phage tail fiber gene and genetically modified high molecular weight (HMW) bacteriocins that are made up of phage tail fiber proteins. The genetically engineered molecules may have multiple specificities and increased binding capabilities, both in number and in strength, to bacterial receptors, thus they are more efficient antimicrobials than their naturally occurring counterparts. Modified HMW bacteriocins may comprise several different phage tail peptide sequences with each sequence having independent binding capabilities. Phage tail peptides may be collected from the engineered bacteriophages. Also transfected host bacteria cells that contain genetic sequences for modified HMW bacteriocins may produce the molecule. In addition to bactericidal effects, pathogenic bacteria affected by these molecules may exhibit some immunostimulating properties. Their virulence is completely or partially neutralized but they remain immunogenic. Modified HMW bacteriocins are viable as topical antibacterials for burn wound infections.

The enzymes bacteriophages produce are antibacterial on their own, thus treatment regimens can be developed where no actual phages are present but the antibacterial effects expected in a bacteriophage infection are observed. Donovan *et al.* [357] extracted bacteriophage lysins from *Clostridium perfringens* phages CP26F and CP390. These enzymes can administered topically with a suitable carrier and perform lytic activity on a common wound pathogen, *C. perfringens*. Moreover the treatment may include other antibiotics including but not limited to vancomycin and neomycin to obtain a better curative effect.

Natural bacteriophage enzymes can be altered by means of DNA recombination or chemical synthesis to create more efficient forms of phage enzymes. More preferably the superior enzyme may be developed by chimerization or shuffling of different enzymes which results in what is called “chimeric” or “shuffled” enzyme structures that possess more desirable traits such as having multiple active sites, binding and catalytic domains. Chimeric enzymes may contain two or more fragments of the same or different phage enzymes thus the enzyme may be more efficient on target molecules or show activity towards various molecules. Shuffled enzymes have increased amounts of binding and catalytic domains acquired from a single phage or multiple bacteriophages thus have an increased activity on the desired substrate. Fischetti *et al.* [358] patented compositions comprising natural, shuffled and chimeric holin and lytic phage enzymes. The composition is proposed to be delivered to burn wounds with bandages as carriers and acts both therapeutically and prophylactically against Staphylococcal and Pseudomonal infections.

The chimerization process can also be carried out between phage structural and catalytic proteins. All bacteriophage families in the *Caudovirales* order have tail-associated murein-degrading enzymes (TAME) that degrade the cell wall of the infected bacteria before new virions are released from the cell. TAMEs have murein-degrading capabilities regardless of the phage reproductive cycle and thus can be purified to exhibit enzymatic activity against bacterial cell walls without an actual phage infection. Padmanabhan *et al.* [359] purified a TAME, ORF56, from Staphylococcal myovirus K, by removing peptide sequences that were restricting its potency as a murein-degrading enzyme that can be used as an antimicrobial. Moreover the purified TAME can be chimerized with the Staphylococcal metalloendopeptidase lysostaphin cell wall binding domain for increased stability and bactericidal activity. The chimera can be topically used in burn wounds against Staphylococcal infections, including methicillin and vancomycin resistant strains of *Staphylococcus* species. The formulation can also be incorporated to wound dressings for prophylactic care of burn wounds.

16.2. Predatory and Probiotic Bacteria

“Bdellovibrio and like organisms” (BALO) typified by *Bdellovibrio bacteriovorus* and *Micavibrio aeruginosavorus* are small Gram(−) bacteria that are of predatory nature against other Gram(−) bacteria species [360]. BALO act as parasites inside bacteria cells and infected bacteria are lysed from the inside. The hostile nature of BALO towards bacterial species such as *Pseudomonas*, and *E.coli* allow these organisms to be used therapeutically in burn wounds. BALO are believed to be effective so long as the infecting bacteria are Gram(−). Williams *et al.* [361] formulated a method for the treatment and prevention of infections caused by bacteria susceptible to BALO attack. In addition to predatory bacteria, Williams *et al*. included bacteriophages in their treatment regimen and observed synergistic effects in treating infections. Phages or BALO were not as effective separately as they were in combination. Gram(−) bacteria can develop predation resistance via phenotypic plasticity to BALO [362] and/or phage resistance through other mechanisms [363]. These phenomena may account for the enhanced remedial effects of BALO and bacteriophage combinations.

Filutowicz [364] patented genetically modified bacteria that contain lethal plasmids which are transmissible through conjugation as shown in (Fig. (12). The invention provides different species of bacteria that can act as antimicrobials by transferring said lethal plasmids to pathogenic strains of the same species. One of the these lethal plasmids expresses a gene that produces deleterious end products to the recipient, while the other results in uncontrolled replication of the plasmid ultimately resulting in cell death. The occurrence of both these adverse effects is prevented in donor cells thus the produced "antibacterial bacteria" are conserved and can function for some time. The plasmid which initiates "runaway" replication inside the pathogen kills bacteria by occupying the replisomes of the pathogen for plasmid replication, thus preventing them being used for chromosomal replication. Compared to conventional antibiotic resistance, development of resistance against these plasmids is more difficult because a multitude of different mutations are required, whereas antibiotic resistance may be acquired more easily. The other plasmid which inserts a killer gene into the pathogen may contain inhibitors for several different key metabolic reactions of the pathogenic bacteria. These genes may be, including but not limited to, sequences that produce non-hemolytic amino acid oligomers which are RNA polymerase inhibitors or RNA molecules that bind to key active sites. The invention can be administered topically to burn wounds or wounds in general and be used in treating or preventing infections caused by common burn wound pathogens such as *P. aeruginosa* and *S. aureus*. Shankar *et al*. [365] carried out an experiment using the aforementioned conjugation method against multidrug resistant *A. baumannii* infections in mice burn wound models. Single dose treatment reduced the number of *A. baumannii* cells in the wound.

Probiotic bacteria are "healthy bacteria" found in many fermented foods and the human bacterial flora, mainly intestinal flora. The loss of these organisms results in the multiplication of pathogenic bacteria inside the human body due to the absence of competition for resources. Moreover probiotic bacteria species have been proven useful in inhibiting pathogenic bacterial growth [366, 367] by producing bacteriocins and other antimicrobial compounds. Thus these mutually beneficial organisms act therapeutically when placed on an infection site. Farmer [368] patented a strain of *Bacillus coagulans* beneficial for remedial use. *B. coagulans* produce coagulin, a bacteriocin-like substance, and lactosporin, an antimicrobial peptide, thus the species is viable as antibacterial probiotics [369]. A variety of topical compositions; gels, creams, lotions, may include cells, spores or products of *B. coagulans* for burn wound treatment and be of therapeutic use against pathogens such as *Pseudomonas* and *Staphylococcus* species.

Borquez *et al.* [370] formulated microencapsulated probiotic *Lactobacillus* spp., primarily *Lactobacillus acidophilus*, for therapeutic use in wound infections including burn wounds. The bacteria are released from the microcapsule as the gelatin matrix composition inside the capsule changes from gel to liquid state. A study done by Borquez *et al*. using said invention in a mouse burn wound model where the burn wound was a scald burn, at 90°C and 10% TBSA. When the formulation was used prophylactically before inoculation and therapeutically upon inoculation of pathogenic bacteria to the wound site, it showed 95% survival against *P. aeruginosa* infection.

16.3. Protozoa

Amoeba, a genus belonging to the kingdom of protozoa, is a eukaryotic unicellular organism. The genus has many pathogenic species as well as species that are not mentioned as harmful in modern medical literature. *In vitro* cultures of amoeba species grow by feeding on bacteria thus amoeba have been explored as a possible antibacterial treatment. Filutowicz *et al*. [371] claim certain species of amoeba can be used in treatment of infections caused by bacteria, even multiple drug resistant strains. These species cause no undesirable effect on humans but consume the pathogenic bacteria in the infection site promoting healing. In essence, non-pathogenic amoeba species act like human phagocytes; both being mobile and having pseudopods, The consumption of the bacteria occurs inside the amoebal cells, thus no cellular residue of dead bacteria is left at the infection site; whereas conventional antibiotics can produce such residues and may contribute to the development of conditions such as endotoxic shock. The phagocytic activity of amoebae is effective against both free bacteria and bacteria that have formed biofilms. The invention focuses on, but is not limited to, *Dictyostelium discoideum* species. Various amoebal species can be included in the treatment regimen achieving increased therapeutic activity and decreased possibility of resistance development. The treatment is also not limited to the application of amoeba; conventional antibiotics can also be incorporated in the regimen. A study conducted on *K. pneumoniae* and MRSA wound infections by the inventors concluded that amoebae were suitable for both preventive measures and curative therapy in burn wounds.

17. CURRENT AND FUTURE DEVELOPMENTS

The present review (taken together with our previous review on this subject [13]) have gone some way to underline the remarkable degree of innovation and invention that exists in the modern day biomedical community. Many of the approaches covered above have been championed by small and start-up biotech companies that have staked their future on trying to contribute to the defeat of a problem that is facing the whole of society. Certainly SBIR funding (small business innovation research) by US agencies such as the National Institutes of Health has been partly responsible for this thriving upsurge in inventions and the generation of intellectual property. How many of these approaches will be translated into actual clinical application remains to be seen in the future. Nevertheless it appears that the growing problem of antibiotic resistance is not going unchallenged, and that the impressive scientific creativity of researchers and entrepreneurs may in the future be up to the task of defeating the threat to mankind posed by uncontrollable antibiotic resistance.

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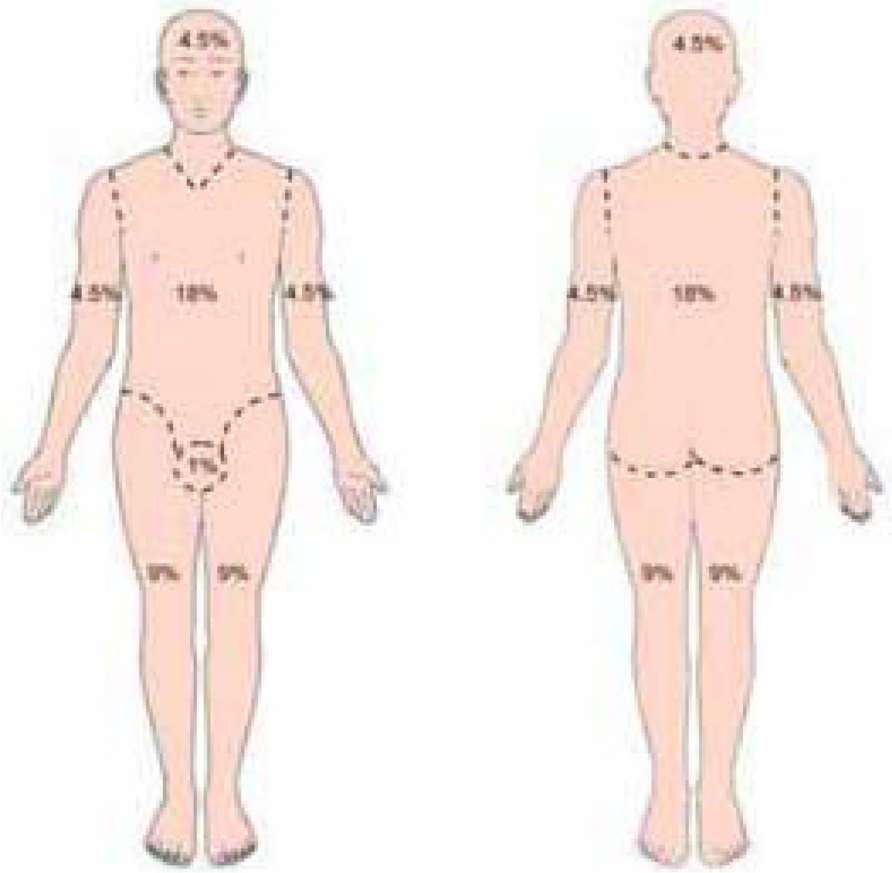

Fig. 1. Rule of nines

Rule of nines is common method of assessing the percentage of total body surface area (TBSA) that got burned. The sum of the corresponding percentages of the burned areas provides a reasonable estimate of the amount of TBSA that got burned.

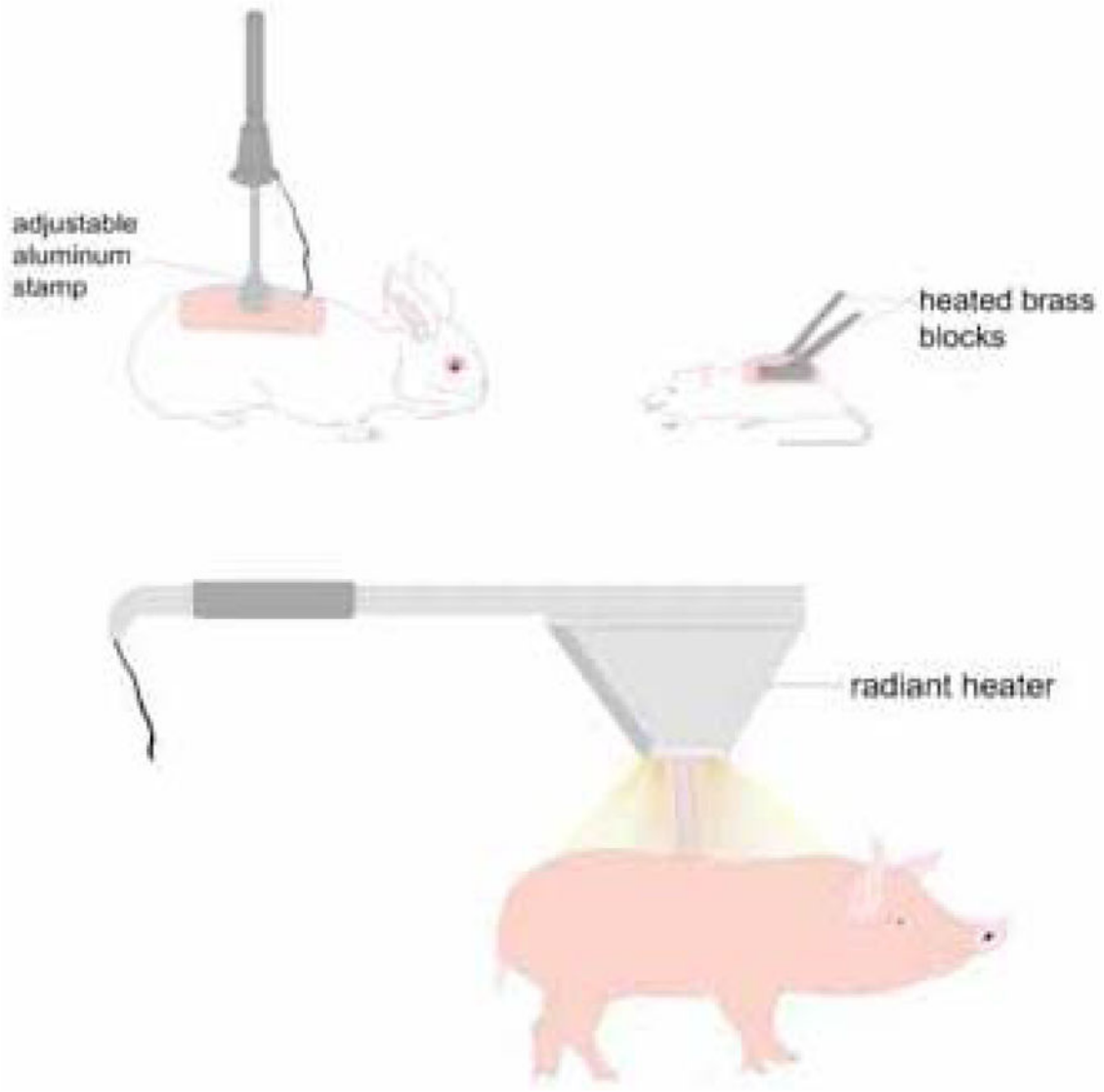

Fig. 2. Three examples of animal burn models

(**A**). Diagram of a rabbit burn model. The burn wound is created by applying heat for 14 seconds from an aluminum soldering iron heated to 80°C to the shaved skin of rabbit. (**B**). Diagram of a murine burn model. The burn wound is created by applying 5 seconds of heat from pre-heated brass blocks at 92–95°C to the shaved skin on both sides of mouse near the elevated skin folds. (**C**). Diagram of a porcine burn model. The burn wound is created by applying 20 seconds of heat from 400°C hand-held radiant heater to the shaved skin of pig.

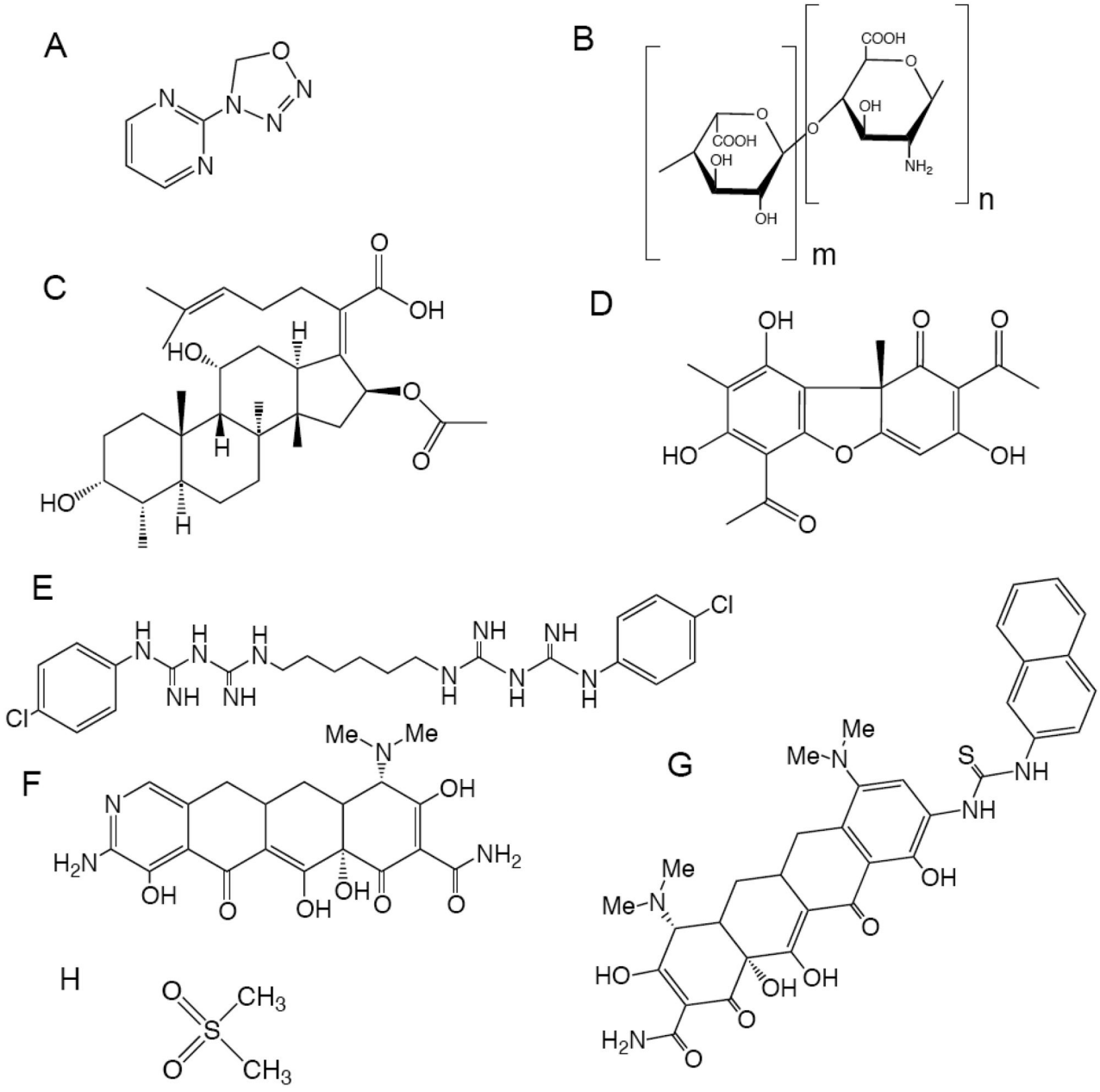

Fig. 3. Structures of some antibiotics, antimicrobial compounds and drug potentiators

(**A).** Firmocidin. (**B**). Alginic acid (alginate). **(C).** Fusidic acid. (**D**). Usnic acid. (**E**). Chlorhexidine. (**F**). 8-aza tetracycline. (**G**). 9-substitued minocycline. (**H**). Methylsulfonylmethane (MSM).

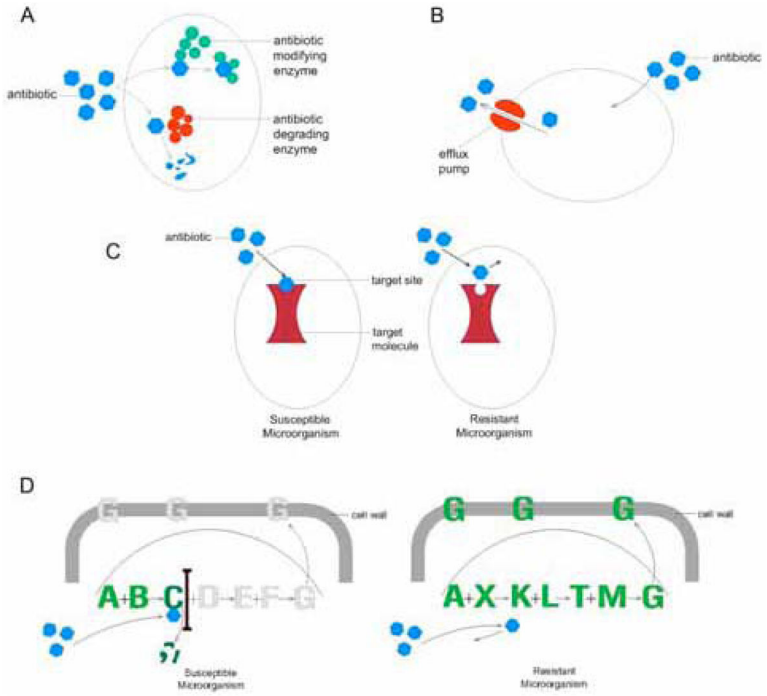

Fig. 4. Four mechanisms of antibiotic resistance

(**A**). Drug inactivation by degradation or modification of the drug. (**B**). Alteration of the target site of the molecule targeted by the drug. (**C**). Reduced drug accumulation in the microorganism. (**D**). Alteration of the metabolic pathway involving the target molecule.

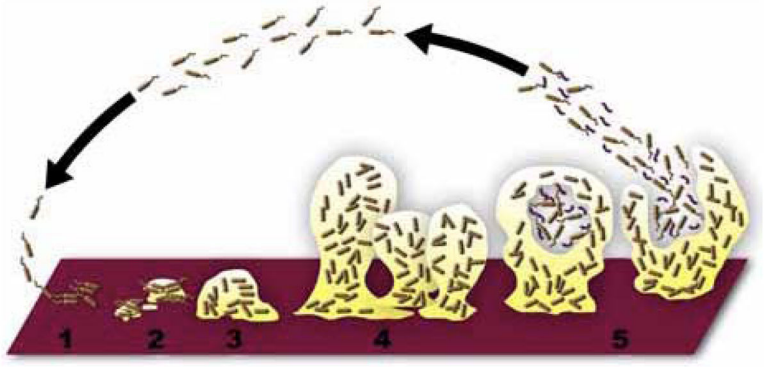

Fig. 5. Life stages of a bacterial biofilm

(1) Settling down. (2) Attachment. (3) Matrix synthesis. (4) Stratification. (5) Dead zones develop. (6) Complete dissolution.

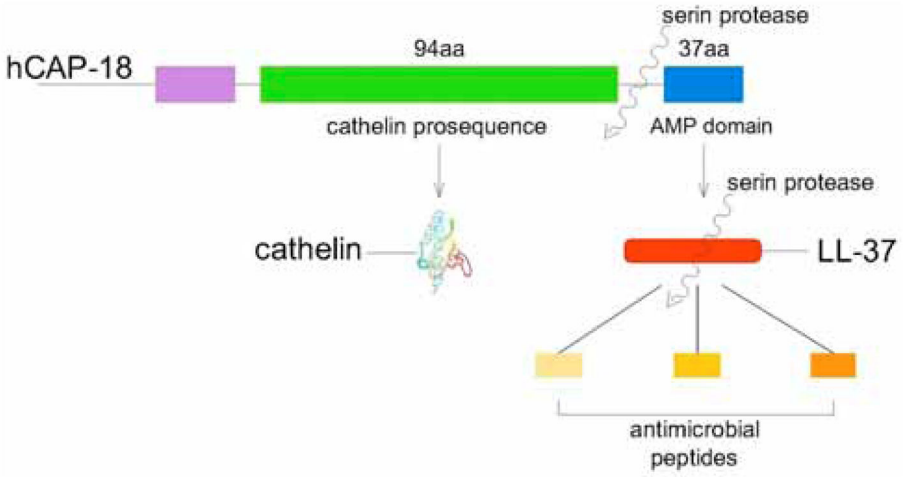

Fig. 6. The antimicrobial peptide, hCAP-18, and its post-translational modifications

hCAP-18 is synthesized in pre-pro-peptide form comprising a signaling peptide, a cathelin-like domain and an antimicrobial peptide (AMP) domain. The initial structure is broken down by signal peptidase and serin proteases. Cathelin-like domain functions as an AMP and protease inhibitor. The AMP domain is called LL-37 due to its two initial aminoacids being leucine and its 37 amino acid residue length. It functions as an AMP and an immunomodulatory agent. LL-37 itself can be further cleaved into various other AMPs.

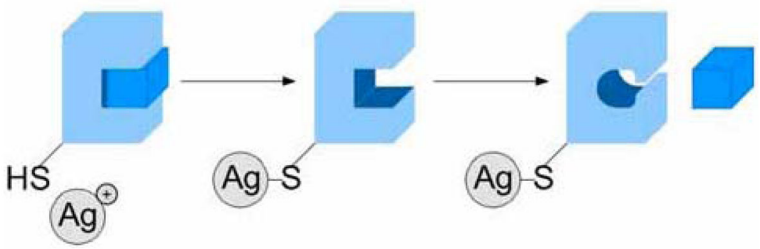

Fig. 7. An example of mechanism of action of silver ions

Silver ions are able to bind thiol functional groups in many essential enzymes. The binding process causes a change in the conformational structure of the enzyme which may change its active site and deactivate it.

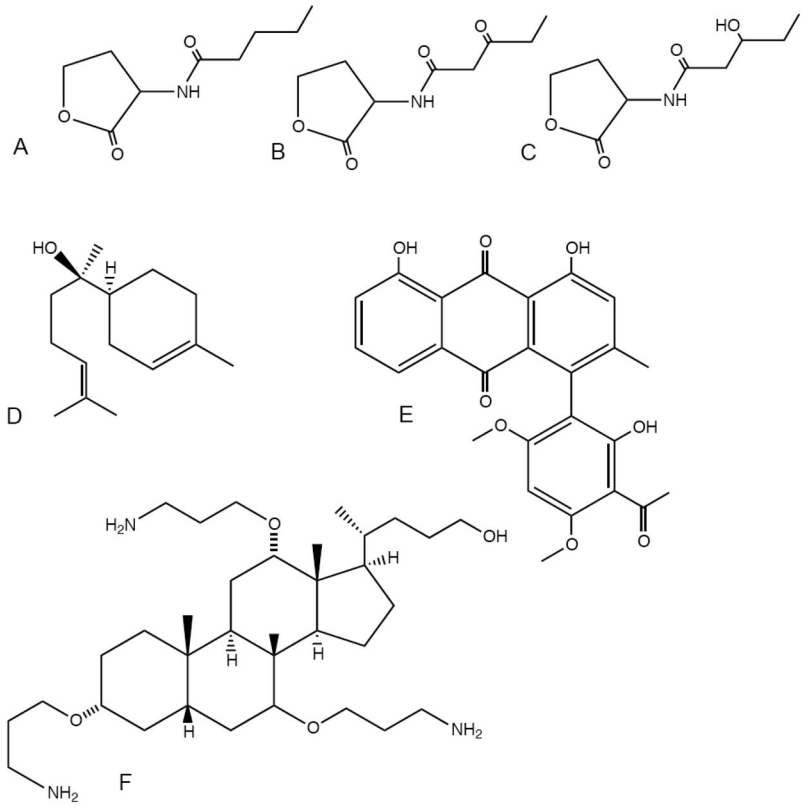

Fig. 8. Structures of bacterial signal molecules involved in quorum sensing, antimicrobial compounds in some botanical extracts and another antimicrobial compound

(**A**), (**B**), (**C**). Homoserin lactone molecules. (**D**). Alfa-bisabolol found in *Cunninghamella* extract. (**E**). Knipholone found in *Bulbine* extract. (**F**). Ceragenin

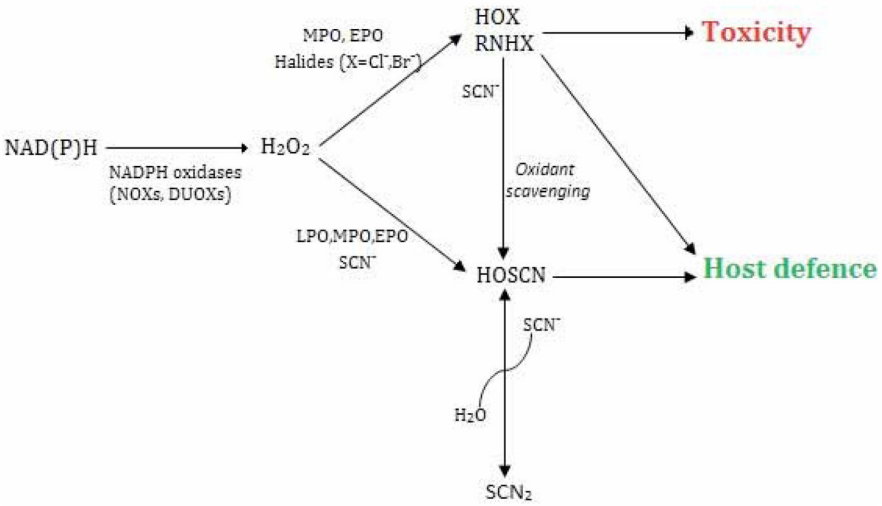

Fig. 9. Host oxidative enzyme mediated antimicrobial pathways

The activity of enzymes such as NADH(P) oxidase, lactoperoxidase (LPO), myeloperoxidase (MPO), and eosinophil peroxidase(EPO) can be potentiated by addition of salts such as chloride, bromide, iodide, and thiocyanate.

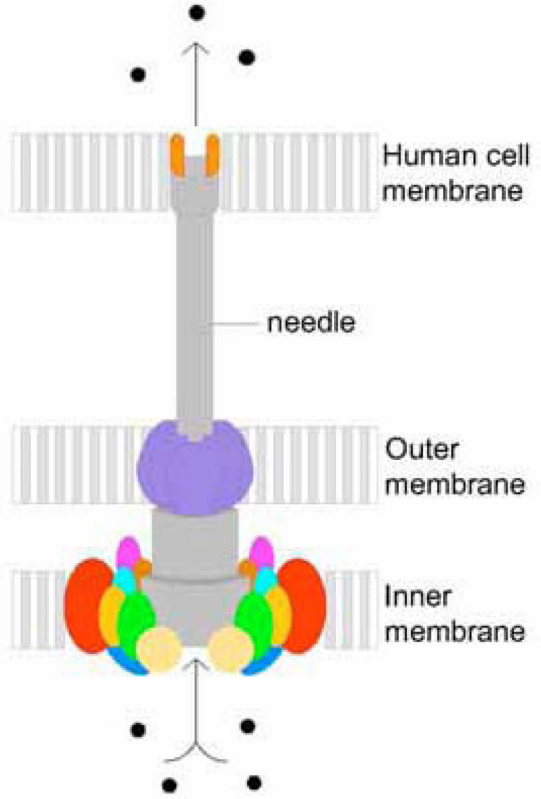

Fig. 10. Type III secretory system

In pathogenic bacteria, the needle-like structure is used as a sensory probe to detect the presence of eukaryotic cells and secrete proteins that help the bacteria infect them.

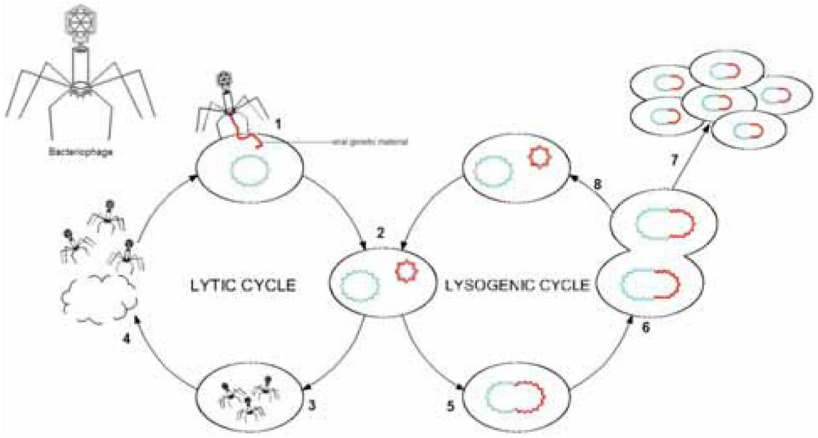

Fig. 11. Bacteriophage reproductive cycle. (1)

Bacteriophage attaches itself on to a receptor of a specific bacterium and injects it genetic material into the cell. **(2)** Genetic material settles inside the cell and the method of replication is determined. **(3)** Lytic reproductive cycle is carried out by the replication of the genetic material and using the ribosomes of the host cell and creating phage proteins which then are combined to make new virions. **(4)** The new virions break open the cell as they leave the host, consequently killing the host bacteria. **(5)** Lysogenic reproductive cycle commences by the integration of viral genetic material to the host genome. **(6)** The integrated genome participates in the replication of bacterial genetic material. **(7)** New generations containing the viral genetic material are produced as a result of cellular division of the host bacteria thus the number of bacteriophage infected cells increase. **(8)** The integrated viral genetic material may disintegrate itself from the genetic material of bacteria due to various reasons such as host conditions. Afterwards the reproductive cycle may change or stay the same depending on the conditions.

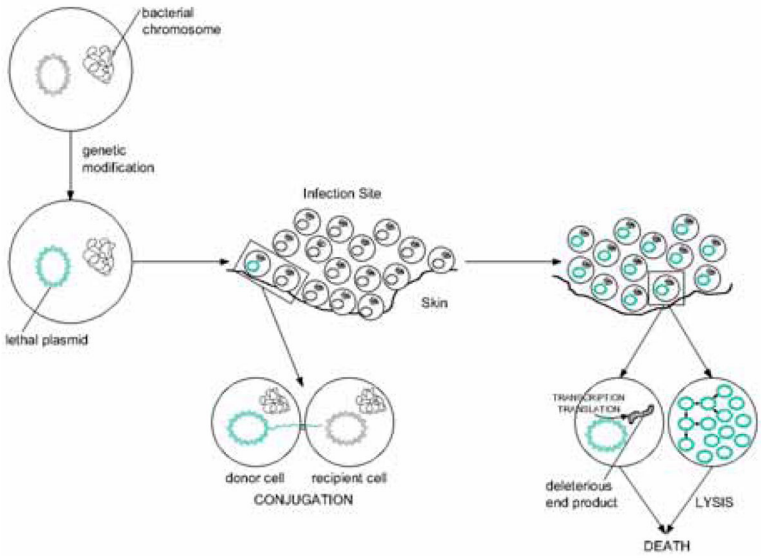

Fig. 12. Mechanism of action of antimicrobial plasmid conjugation

A non pathogenic bacterium is genetically modified to contain a “lethal plasmid” that performs antimicrobially towards other pathogens but not the host. The bacterium is placed in a place where pathogenic colonization is evident, a bacterial biofilm on the skin is given as an example. The plasmid is conjugated between the genetically modified and pathogenic bacteria. Over time the whole colony obtains the therapeutic plasmid. Microorganisms that have received the plasmid express its deleterious products and are lysed because of their damage. Other recipients fail to perform cellular division and die due to the plasmid occupying the necessary enzymes as it is engineered to replicate itself excessively.

Table 1

List of Numerous Burn Wound Pathogens.

Group		Species	Abundance in Burn Wound İnfections (%)
BACTERIA	Gram +	*Staphylococcus aureus*	6.5–37.6 [372–377]
		Meticillin-resistant *S.aureus (MRSA)*	0.2 [375]
		Staphylococcus epidermidis	1.4–2.9 [376]
		Enterecoccus spp.	1.6–11.0 [372, 375, 377]
		Vancomycin-resistant enterococci	N/A
		Clostridium spp.	0.8 [372]
		Beta-hemolytic streptococci	13.6 [374]
		Coagulase-negative streptococci	0.7–23.4 [374, 375]
		Peptostreptococcus spp.	7.7 [372]
		Propionibacterium acnes	1.6 [372]
		Streptococcus pyogenes	1.1 [372]
		Bacillus spp.	1.3 [372]
		Lactobacillus spp.	0.3 [372]
		Corynebacterium spp.	4.3 [375]
	Gram −	*Pseudomonas aeruginosa*	17–51.5 [372–377]
		Escherichia coli	2.9–8.6 [372–374, 377]
		Klebsiella pneumoniae	2.6–10.6 [372, 374, 377]
		Serratia marcesens	2.1–6.5 [373, 375, 377]
		Enterobacter spp.	1.3–10.6 [372–375]
		Proteus spp.	2.3–16.1 [372, 373, 376]
		Acinetobacter baumannii	3.2–9.9 [373, 375]
		Bacteriodes spp.	16.5 [372]
		Veilonella sp.	1.3 [372]
		Aeromonas hydrophilia	1.4 [375]
FUNGI		*Fusobacterium* spp.	1.3 [372]
		Candida spp.	1.3–22.4 [373, 377, 378]
		Aspergillus spp.	3.1 [378]
		Fusarium spp.	N/A
		Alternaria spp.	N/A
		Rhizopus spp.	N/A
		Mucor spp.	N/A
		Zygomycetes	0.8 [378]
		Penicillium spp.	0.5 [378]
VIRUSES		Herpes simplex virus (HSV)	N/A
		Cytomegalovirus (CMV)	N/A
		Varicella-zoster virus (VZV)	N/A

N/A not available

Table 2

List of Some Topical Antibiotics Used for Burn Wound Infections.

Antibiotic	Spectrum	Mechanism of Action	Important Information
Aminoglycoside (e.g.Neomycin)	Activity against aerobic Gram− bacteria bacilli and Gram+ aerobes No activity against anaerobes [379]	Inhibition of protein synthesis	
Bacitracin	Gram+ bacteria particularly Staphylococci and beta-hemolytic streptococci No activity on against Gram− bacteria [12]	Disruption of cell wall	
Mafenide acetate	Broad bacteriostatic activity against Gram− bacteria especially P. aeruginosa Little activity against Gram+ bacteria such as S.aureus [9]	Unknown. Reduces the bacterial load.	More suitable for non-facial burns compared to bacitracin [380] It is converted to a substance that is carbonic anhydrase inhibitor, thus causing metabolic acidosis [9]
Mupirocin	Inhibitor against Gram+ skin flora [381, 382] High activity against streptococci and staphylococci including MRSA No activity against enterococci Little activity against Gram− bacilli and anaerobes [12]	Inhibition of protein synthesis	It and its combinations with some other antimicrobials have been observed to be effective against MRSA [383]
Neosporin (bacitracin + neomycin + polymyxin B)	Combined spectrum of bacitracin, neomycin and polymyxin B		The bactericidal effect is mainly
Nitrofurazone	Activity against Gram− and Gram + bacteria	Inhibition of enzymes	
Nystatin	Fungi especially Candida	Disruption of the fungal cell wall (by binding to ergostreol, a distinctive component of fungal cell wall)	Should be used in combination with antibacterials for burn wounds
Polymyxin B	Effective against Gram− bacteria No activity against Gram+ bacteria [12]	Disruption of the cell wall	Prevents polymorphonucleer cells from killing of the phagocytosed microorganisms [384]
Rifampin	Activity against most Gram+ bacteria including streptococci and especially staphylococci and some Gram− bacteria [385]	Inhibition of RNA synthesis	
Tetracycline	Activity against many Gram− and Gram+ bacteria [386]	Inhibition of protein synthesis	Aggravating bacterial resistance
Colistin (Polymyxin E)	Activity against selected Gram− bacteria including Acinebacter, Pseudomonas aeruginosa, Klebsiella and Enterobacter [387]	Disruption of cell membrane integrity [388]	

Table 3

The Effects of Firmocidin on Various Microorganisms.

Affected Microorganism	Firmocidin Effect
Group A Streptococcus	Bactericidal
Group B Streptococcus	Bacteriostatic
S. aureus	
MRSA	Growth inhibition

Data based on information contained in patent [389]

Table 4

Novel Patented Tetracycline Formulations.

Name	Patent No.	Composition	Additional Information
Foamable topical tetracycline composition	[390]	0.1–10% wt tetracycline + 60–95% wt oil + oily emollient + 0.01–15% surfactant + 0.01–10% foam adjuvant	
Topical hydrophobic breakable tetracycline composition	[391]	60–99% wt hydrophobic oil +viscosity modifying agent + tetracycline	More than 90% of the tetracycline in the composition stays chemically stable for at least 6 months. Is packaged in a breakable foam.
8-aza tetracycline compounds	[392]		New tetracycline analog having imporoved efficacy antibacterially.
Novel deuterated and florinated tetracycline analog	[393]		Incerased bond strength between carbon (C) and deuterium compared to C and hydrogen (H). Useful for both Gram+ and Gram− bacteria including MRSA
9-substitued minocyline	[394]		Minocycline is the most lipid-soluble tetracycline [395]

wt: by weight

Table 5

List of AMPs Covered in the Previous Review.

Name of Peptide	Therapeutic Effects	References
Cecropin	Antimicrobial, some fragments accelerate wound repair	[396–398]
D2A21 (Demegel)	Antimicrobial, mechanism not disclosed	[399, 400]
Histone H1.2	Antimicrobial, binds with DNA and cellular membranes	[401, 402]
rBPI2l	Down regulation of CD14	[403, 404]

Table 6

List of Patented Iodine Compositions with Burn Wound Applications.

Name	Patent No.	Composition
Povidone-iodine	[405]	5% by weight wt Povidone-iodine + 20% wt sugar
	[406]	0.4–12.5% wt Povidone-iodine + non-steroidal/steroidal anti-inflammatory drug
	[407]	Povidone-iodine + sucrose + gelling agent
Liposomal iodine (Repithel)	[408]	Dry povidone-iodine encapsulated in liposome
Cadexomer iodine (Iodosorb)	[409]	Hydrophilic starch powder containing 0.9% wt iodine
Other	[410]	An iodophore 0.1–2% wt supplying iodine + a monosaccharide and/or sugar alcohol
	[411]	12% wt molecular iodine + 30% wt non-ionic surfactant + iodine-solubilizing halide ion

wt: by weight

Table 7

List of Various Natural Molecules and Extracts with Burn Wound Applications.

Product		Antimicrobial Structure	Spectrum of Activity	Additional Information
Alcoholic extract of fungi of genus *Cunnighamella* [412]		Methanol and/or ethanol	Gram− and Gram+ bacteria, filamentous fungi; particularly*Aspergillus fumigatus, Penicillium expansum, Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Escherichia coli* and *Salmonella typhimuium.*	• It can also be used as a wound healing agent. • Adenosin constituent of the extract show high antimicrobial effect against *S. aureus*.
Essential oils	From *Matricaria chamomilla* flowers [413]	A-bisabolol (a sesquiterpenic alcohol)	(in combination with an ozonized oil) *Pseudomonas*, beta-hemolytic *Streptococcus*, *Staphylococcus*, *Klebsiella* and other pathogen bacteria	• The combination of the essential oil and an ozonized oil show synergistic bactericidal and cicatrising effect with reduced ROS production.
	From Blue Curls (*Trichostema lanatum*) var. Pirzada [414]	Terpinen-4-ol	Strong activity against Gram+ bacteria Less effective against Gram− bacteria	• The extract consists of 60% terpinen-4-ol. • Terpinen-4-ol inhibits the infections associated with first-and-second degree burns and prevent scarred healing.
	Tea tree oil extract [414, 415]	Terpinen-4-ol		• Terpinen-4-ol inhibits the infections associated with first- and-second degree burns and prevent scarred healing.
Bulbine fruitescens extracts [416]		Knipholone	*S. aureus*, including MRSA and clindamycin, erythromycin and ciprofloxacin-resistant *S. aureus*	• The patented composition of the reference requires the juice of plant's leaves to be treated with hydrogen peroxide. • Hydrogen peroxide and B. fruitescens extract show synergism. • Curative for burns.
Eremophila longifolia extracts [417]			Mainly Gram+ bacteria such as *S. aureus*, *Staphylococcus epidermidis*, Streptococcus and Gram− bacteria involving *Serratia marcescens*, P. [418]	• It is suitable for topical application on burn wound infections.
Tamarindus indica seed polysaccharide [419]		Polysaccharide	Certain Gram+ and Gram− bacteria	• Antimicrobial effects are observable especially in topical use. • Xylitol and Fluoride have been found to be synergistic.

Table 8

List of Various Patented Photosensitizers.

Molecule Group	Photosensitizer Name
Acridine	Acridine yellow [420]
Benzophenoxazinium dyes	Unnamed derivative
Δ-aminolevulinic acid	Δ-aminolevulinic acid [421]
Flavins	Riboflavin [422]
Fullerenes	Cationic fullerene [423]
Phathalocyanin dyes	Unnamed derivatives [424]
Phenothiazine dyes	Dimethylmethylene blue [425]
	Methylene blue [426]
	New methylene blue
	Toluidine blue O [427]
Quinones	Menaquinone [422]
	Phylloquinone [422]
Tetrahydropyrroles/porphyrins	XF-73 [428]
	Cobalamin [422]
	Unnamed derivatives

Table 9

List of Novel Bacteriophages and Corresponding Phage Peptides Developed by Garcia *et al*.

Bacteriophage/Peptide name	Target bacteria
F391/08	*Klebsiella pneumoniae*
F387/08	*Klebsiella pneumoniae*
F394/08	*Acinetobacter baumannii*
F488/08	*Escherichia coli*
F510/08	*Pseudomonas aeruginosa*
F44/10	*Staphylococcus aureus*
F125/10	*Staphylococcus aureus*